STUDY TITLE
ASTM E2197-11 Standard Quantitative Disk Carrier Test Method

Study Identification Number
GLP1374

Protocol Number
P1448

Product Identity
Decon 7 Part 1
Lots:16-13, 16-14, and 16-15
Decon 7 Part 2
Lots: 16-16, 16-17, and 16-18
Booster
Lot: 470572802

Test Microorganism
Clostridium difficile ATCC 43598 (endospores)

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

Author
Eric Cuellar, B.S.
Study Director

Study Completion Date
03OCT2016

Testing Facility
Microchem Laboratory
1304 W. Industrial Blvd.
Round Rock, TX 78681

Study Sponsor
Joe Drake
Decon7 Systems, LLC
7575 E. Redfield Rd., Suite 235
Scottsdale, AZ 85260

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: _____

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.

Study Director

Company: Microchem Laboratory

Name: Eric Cuellar, B.S.

Title: Study Director

Signature: Eric Cuellar Date: 04 Oct 2016

Study Sponsor

Company: Decon7 Systems, LLC

Name: Joe Drake

Title: Study Sponsor

Signature: _____ Date: _____

Submitter

Company:

Name:

Title:

Signature: _____ Date: _____

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QUALITY ASSURANCE STATEMENT

Study Title: ASTM E2197-11 Standard Quantitative Disk Carrier Test Method

Study ID#: GLP1374

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	26FEB2016	29FEB2016	29FEB2016
Draft Report	28SEP2016	28SEP2016	29SEP2016
Final Report	03OCT2016	03OCT2016	03OCT2016

Quality Assurance Unit:

Signature: Travis Chesser Date: 03OCT2016

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

PERSONNEL INVOLVED IN THE STUDY

Study Director

Name: Eric Cuellar, B.S.
Company: Microchem Laboratory
Title: Study Director

Scientific Director

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

Assisting Personnel

Name: Diego Ugarte, B.S.
Company: Microchem Laboratory
Title: Technician

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FINAL STUDY REPORT SUMMARY

Study Title
ASTM E2197-11 Standard Quantitative Disk Carrier Test Method

Study Identification Number
GLP1374

Protocol Number
P1448

Test Microorganism
Clostridium difficile ATCC 43598 (endospores)

Study Sponsor
Joe Drake
Decon7 Systems, LLC
7575 E. Redfield Rd., Suite 235
Scottsdale, AZ 85260

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

Study Director
Eric Cuellar, B.S.

Study Completion Date
03OCT2016

Study Objective
To determine, using the ASTM E2197-11 Standard Quantitative Disk Carrier Test Method, the sporicidal efficacy of Decon7 Part 1, Decon7 Part 2 and booster. The products were combined at a ratio of 1 part Decon7 Part 1, 1 part Decon7 Part 2 and 2% v/v booster. The products were tested against *C. difficile* ATCC 43598 (endospores) supplemented with an artificial soil load at a contact time of 10 minutes ± 5 seconds and a test temperature of 20-25°C.

Study Conclusion in Brief
Decon 7 Part 1 (lot: 16-15), Decon 7 Part 2 (Lot: 16-18) and booster (Lot: 4705722802) combined at a ratio of 1 part Decon 7 Part 1, 1 part Decon 7 Part 2 and 2% v/v booster met the protocol-specified success criteria when tested against *C. difficile* ATCC 43598 (endospores) supplemented with an artificial soil load at a 10 minute ± 5 seconds contact time and a test temperature of 20-25 °C. Decon 7 Part 1 (lots: 16-13, 16-14), Decon 7 Part 2 (Lots: 16-16, 16-17) and booster (Lot: 4705722802) combined at a ratio of 1 part Decon 7 Part 1, 1 part Decon 7 Part 2 and 2% v/v booster did not meet the protocol specified success criteria when tested at the aforementioned parameters.

FINAL STUDY REPORT

Important Dates

Study Initiation Date: 23FEB2016
Experimental Start Date/Time: 24FEB2016/1312
Experimental End Date/Time: 02MAR2016/1613

Test Substance Information

Name: Decon7 Part 1

Lots: 16-13, 16-14, 16-15
Alkyl Dimethylbenzyl Ammonium Chloride, (wt.%) – 3.08%, 3.06%, 3.09% respectively
Date of Manufacture: 04JAN2016
Date Received: 20JAN2016
Expiration Date: 14JAN2017

Name: Decon7 Part 2

Lots: 16-16, 16-17, 16-18
H_2O_2, (wt.%) – 7.604%, 7.586%, 7.584% respectively
Date of Manufacture: 04JAN2016
Date Received: 20JAN2016
Expiration Date: 14JAN2017

Name: Booster

Lot: 4705722802
Inert Ingredient

Storage Conditions: Ambient temperature under fluorescent lighting
Form: Multi-part liquid product
Mixing ratio: 1 part Decon 7 Part 1, 1 part Decon 7 part 2, 2% v/v booster

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FINAL STUDY REPORT (CONT.)

Test Parameters

Microorganism:	*Clostridium difficile* ATCC 43598 (endospores)
Number of Test Carriers:	10 Carriers per Lot
Number of Control Carriers:	3 Carriers per Lot
Carrier Type:	1.0 cm diameter stainless steel disks
Contact Time:	10.0 minutes ± 5 seconds
Neutralization Broth:	Dey engley (D/E) broth supplemented with 0.1% catalase
Organic Soil Load:	3-part soil load consisting of bovine serum albumin, yeast extract, and mucin
Carrier Inoculation Volume:	0.010 ml
Carrier Dry Time:	30 ± 5 Minutes in a biological safety cabinet
Carrier Vacuum Time:	2 Hours Carriers were stored in a desiccator without vacuum until use in the test, all carriers were used within 24 hours of drying
Incubation Temperature:	36 ± 1°C
Incubation Time:	48 ± 4 hours for control plates 72 ± 4 hours for treated plates
Additional Incubation Time:	An additional 48 ± 4 hours if few or no colonies were observed after initial 72 hour incubation period
Incubation Conditions:	Anaerobic
Test Temperature:	20 – 25°C
Test/Control Substance Volume:	0.05 mL
Control Substance:	Phosphate Buffered saline (PBS) with 0.1% tween 80
Membrane Filters for Enumeration:	47 mm diameter 0.2 μm Polyethersulfone (PES)
Plating Medium:	Brain Heart Infusion Agar supplemented with yeast extract, 0.1% sodium taurocholate, and 7.0% defibrinated horse blood
Plating Medium Pre-Reducing Time:	≥ 18 hours

Test Method

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

PROTOCOL CHANGES

Protocol Amendment(s)

No protocol amendments were made for this study.

Protocol Deviation(s)

A deviation from the approved protocol occurred on 26FEB2016, 27FEB2016, and 28FEB2016 wherein Control Carrier enumeration filters were observed to be greater than 200 and were used in calculations for control carrier count results. This deviation was not thought to have impacted the results of the study as filter enumerations were not above 300 CFU per filter and control carrier enumeration results were within the acceptable range listed in the approved protocol.

A deviation from the approved protocol occurred on 26FEB2016, 27FEB2016, and 28FEB2016 wherein Neutralization control filters were observed to be greater than 100 CFU. This deviation was not thought to have impacted the results of the study due to the proximity of neutralization control filters to the protocol specified 100 CFU or less and the validity of the neutralization results observed.

CONTROLS

Enumeration of Inoculated Test Carriers

Following the conclusion of the dry time, dried inoculated carriers were assayed in one set of three for each day of testing. Control carriers were treated with control substance following the treatment and neutralization of test carriers. Each control carrier was individually transferred to a sterile Nalgene vial using flame-sterilized forceps, with the inoculated side facing up. Carriers were treated with 0.050 ml control substance at predetermined staggered intervals. At the end of the contact time, 10 ml of neutralization buffer was added to each Nalgene vial. Vials were then capped and vortex mixed for 30 ± 5 seconds at high speed. Serial dilutions of the resulting neutralized control carriers were performed in Phosphate Buffered Saline supplemented with 0.1% Tween 80 (PBS-T) and the entire contents of tubes representing the appropriate dilutions were filtered, aseptically transferred to plating medium, and incubated alongside treated test carrier enumerations.

"Soil" Sterility Control

An aliquot of each component of test "soil" was plated using sterile growth medium and incubated alongside enumeration plates to verify sterility at the time of test.

Media Sterility Controls

A plate containing growth medium was incubated along side the test to confirm plating media sterility. Independent plates containing 0.100 ml of each PBS, PBS-T and neutralization broth media were incubated along side the test to confirm media sterility.

Neutralization Control

A 0.050 ml aliquot of test substance was added directly to a sterile Nalgene vial containing 10 ml of the test neutralizer, this suspension represented the "neutralization test" suspension. Separately a 0.050 ml aliquot of Phosphate Buffered Saline (PBS) was added directly to a sterile Nalgene vial containing 10 ml of the test neutralizer, this suspension represented the "neutralization control" suspension. The supplemented spore suspension was diluted in sterile PBS supplemented with 0.1% Tween 80 (PBS-T) to a concentration of ≤1000 CFU/1.0 ml. 0.100 ml of the appropriate dilution was added directly to each "neutralization test" and "neutralization control" suspension before suspensions were filtered, plated, and incubated alongside the test to confirm comparable levels of the test microorganism were recovered between "neutralization test" and "neutralization control" suspensions.

Test Microorganism Purity Control (Also Test System Viability Control)

A loopful of each test microorganism used in this study was subcultured to an appropriate growth agar medium and incubated alongside enumeration plates to morphologically confirm the presence of a pure culture at the time of test. Enough test system purity controls were prepared such that one purity control could be included in each anaerobic chamber used in the test to confirm that anaerobic conditions were met during incubation and that the test system was viable.

STUDY ACCEPTANCE CRITERIA

The experimental success (controls) criteria follow:

- Control carriers must demonstrate a mean log density of at least 6.0 (corresponding to $1x10^6$ CFU/Carrier) and not above 7.0 (Corresponding to a mean log density of $1x10^7$ CFU/Carrier.)
- The neutralizer sterility control is negative for growth.
- The carrier sterility control is negative for growth.
- The viability growth control is positive for growth.
- The Test system purity control demonstrates a pure spore suspension.
- The neutralization control incoulum demonstrates ≤100 CFU.
- The "soil" sterility control is negative for growth.
- The media sterility controls are negative for growth.

The EPA performance criterion for disinfection follows:

- A minimum of 6 $\log_{10}$ reduction in viable spores is observed under the conditions tested.

Retesting guidance for disinfection follows:

- When a test fails and the $\log_{10}$ density of the test carriers is below 6.0, no retesting is necessary.
- When a test passes and the $\log_{10}$ density of the test carriers is above 7.0, no retesting is necessary.
- When a test fails and the $\log_{10}$ density of the test carriers is above 7.0, retesting may be conducted.

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CALCULATIONS AND STATISTICAL ANALYSIS

The following calculations were used in the study. Calculation variables may have been adjusted based on volumes and dilutions used.

Test Carrier Enumeration

$$\frac{(\text{Average CFU for } 10^{-X}) + (\text{Average CFU for } 10^{-Y}) + (\text{Average CFU for } 10^{-Z})}{10^{-X} + 10^{-Y} + 10^{-Z}} = \text{CFU/ml}$$

[(CFU/ml) X 10] = CFU/Carrier

Log_{10} density per carrier (treated or control) = $\log_{10}$(CFU/Carrier)

Log_{10} Reduction Calculation

Mean $\log_{10}$ control carriers – mean $\log_{10}$ treated carriers = Log_{10} reduction

NOTE: If no spores were recovered from any treated carriers, log reduction was reported as greater than log_{10} density of control carriers. If more than 200 spores were recovered from treated carriers, log reduction was reported as less than the log_{10} reduction between control and treated carriers.

STUDY RECORD AND TEST SUBSTANCE RETENTION

Study Record Retention

The study report and corresponding data sheets will be held in the archives of Microchem Laboratory for at least 2 years after the date of the final report and then may be destroyed. If the study is used by the Study Sponsor in support of a label claim, documentation may be returned to the Study Sponsor for archiving at Study Sponsor's expense.

- Raw Data
- Documentation
- Records
- Protocols and protocol amendments/deviations
- Final reports and final report amendments
- Correspondence and other documents relating to interpretation and evaluations of data, other than those documents contained in the final report.

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 may be destroyed >30 days after study completion.

RESULTS

The tables that follow were the results from the study:

Table 1. Decon 7 Part 1 (Lot: 16-14), Decon 7 Part 2 (Lot: 16-17) Booster (Lot: 470572802) Product Performance and Carrier Enumeration Results from Testing Conducted on 25FEB2016

Test Microorganism	Contact Time	Test or Control Substance	Carrier Replicate	Carrier Enumeration (CFU/Carrier)	Log_{10} Density	Average Log_{10} Density	Log_{10} Reduction Relative to Control Carrier Counts
C. difficile ATCC 43598 (endospores)	10 Minutes	PBS-T Control Substance	1	2.78E+06	6.44	6.42	N / A
			2	2.27E+06	6.36		
			3	2.82E+06	6.45		
		Decon 7 Part 1 (Lot: 16-14), Decon 7 Part 2 (Lot: 16-17), Booster (Lot: 470572802)	1	0.00E+00	0.00	0.54	5.88
			2	2.00E+00	0.30		
			3	1.20E+01	1.08		
			4	7.30E+01	1.86		
			5	2.00E+00	0.30		
			6	1.30E+01	1.11		
			7	5.00E+00	0.70		
			8	1.00E+00	0.00		
			9	0.00E+00	0.00		
			10	0.00E+00	0.00		

Table 2. Decon 7 Part 1 (Lot: 16-15), Decon 7 Part 2 (Lot: 16-18) Booster (Lot: 470572802) Product Performance and Carrier Enumeration Results from Testing Conducted on 26FEB2016

Test Microorganism	Contact Time	Test or Control Substance	Carrier Replicate	Carrier Enumeration (CFU/Carrier)	Log_{10} Density	Average Log_{10} Density	Log_{10} Reduction Relative to Control Carrier Counts
C. difficile ATCC 43598 (endospores)	10 Minutes	PBS-T Control Substance	1	2.89E+06	6.46	6.46	N / A
			2	2.81E+06	6.45		
			3	2.89E+06	6.46		
		Decon 7 Part 1 (Lot: 16-15), Decon 7 Part 2 (Lot: 16-18), Booster (Lot: 470572802)	1	1.00E+00	0.00	0.22	6.24
			2	0.00E+00	0.00		
			3	0.00E+00	0.00		
			4	1.00E+00	0.00		
			5	1.00E+00	0.00		
			6	4.00E+00	0.60		
			7	0.00E+00	0.00		
			8	3.00E+00	0.48		
			9	1.20E+01	1.08		
			10	1.00E+00	0.00		

RESULTS (Cont.)

Table 3. Decon 7 Part 1 (Lot: 16-13), Decon 7 Part 2 (Lot: 16-16) Booster (Lot: 470572802) Product Performance and Carrier Enumeration Results from Testing Conducted on 24FEB2016

Test Microorganism	Contact Time	Test or Control Substance	Carrier Replicate	Carrier Enumeration (CFU/Carrier)	Log_{10} Density	Average Log_{10} Density	Log_{10} Reduction Relative to Control Carrier Counts
C. difficile ATCC 43598 (endospores)	10 Minutes	PBS-T Control Substance	1	2.43E+06	6.39	6.40	N / A
			2	2.38E+06	6.38		
			3	2.67E+06	6.43		
		Decon 7 Part 1 (Lot: 16-13), Decon 7 Part 2 (Lot: 16-16), Booster (Lot: 470572802)	1	7.00E+00	0.85	0.44	5.96
			2	4.00E+00	0.60		
			3	1.00E+01	1.00		
			4	3.00E+00	0.48		
			5	1.00E+01	1.00		
			6	3.00E+00	0.48		
			7	0.00E+00	0.00		
			8	0.00E+00	0.00		
			9	0.00E+00	0.00		
			10	0.00E+00	0.00		

Table 4. Neutralization Control Results from Testing Conducted on 24 FEB 2016, 25 FEB 2016 and 26 FEB 2016

Date Testing Conducted	Test Substance Evaluated	Neutralization Test Count	Neutralization Control Count	Neutralization Control Result
24 FEB 2016	Decon 7 Part 1 (Lot: 16-13), Decon 7 Part 2 (Lot: 16-16), Booster (Lot: 470572802)	168 CFU	172 CFU	Passed
25 FEB 2016	Decon 7 Part 1 (Lot: 16-14), Decon 7 Part 2 (Lot: 16-17), Booster (Lot: 470572802)	160 CFU	178 CFU	Passed
26 FEB 2016	Decon 7 Part 1 (Lot: 16-15), Decon 7 Part 2 (Lot: 16-18), Booster (Lot: 470572802)	138 CFU	139 CFU	Passed

RESULTS (Cont.)

Table 5. Incubation of Test Materials from Testing Conducted on 24FEB2016, 25FEB2016, and 26FEB2016

Test Substance	Test Materials	Incubation Temperature Range	Total Incubation Duration
Decon 7 Part 1 (Lot: 16-13), Decon 7 Part 2 (Lot: 16-16), Booster (Lot: 470572802)	Control Plates (Control Carrier Enumeration Plates, Sterility Control Plates, and Neutralization Verification Plates)	36±1 °C	47 hours, 22 minutes
	Treated Carrier Test Enumeration Plates		117 hours, 39 minutes
Decon 7 Part 1 (Lot: 16-14), Decon 7 Part 2 (Lot: 16-17), Booster (Lot: 470572802)	Control Plates (Control Carrier Enumeration Plates, Sterility Control Plates, and Neutralization Verification Plates)		49 hours, 42 minutes
	Treated Carrier Test Enumeration Plates		119 hours, 31 minutes
Decon 7 Part 1 (Lot: 16-15), Decon 7 Part 2 (Lot: 16-18), Booster (Lot: 470572802)	Control Plates (Control Carrier Enumeration Plates, Sterility Control Plates, and Neutralization Verification Plates)		48 hours, 39 minutes
	Treated Carrier Test Enumeration Plates		119 hours, 45 minutes

Table 6. Study Controls for testing conducted on 24 FEB 2016, 25 FEB 2016, and 26 FEB 2016

Test System Viability Control	Neutralization Broth Sterility	Plating Media Sterility Control	Artificial Soil Load Sterility Controls	Control Substance Sterility Control	Dilution Media Sterility Control	Test System Purity Streak Control
Growth	No Growth	No Growth	No Growth	No Growth	No Growth	Pure

STUDY CONCLUSION

For Study Identification Number GLP1374, test substance Decon 7 Part 1 (Lots: 16-13, 16-14, and 16-15), Decon 7 Part 2 (Lots: 16-16, 16-17, and 16-18) and Booster (Lot: 470572802) mixed at a ratio of 1 part Decon 7 Part 1, 1 part Decon 7 Part 2, 2% (v/v) booster was tested against *Clostridium difficile* ATCC 43598 (endospores) supplemented with 3 part soil. A total of 10 contaminated carriers were exposed to the test substance for a contact time of 10 minutes ± 5 seconds and then chemically neutralized.

Test substance Decon 7 Part 1 (Lot: 16-13), Decon 7 Part 2 (Lot: 16-16), and booster (Lot: 470572802), mixed at a ratio of 1 part Decon 7 Part 1, 1 part Decon 7 Part 2 and 2% booster (v/v), demonstrated 5.96 Log reduction when tested against *C. difficile* ATCC 43598 (endospores) supplemented with a 3 part organic soil load at a contact time of 10 minutes ± 5 seconds. These lots of test substance did not meet the U.S. EPA Product Performance Guidelines for disinfection of *C. difficile* endospores outlined in EPA Product Performance Test Guidelines, OCSPP 810.2100 or the success criteria detailed in the approved protocol.

Test substance Decon 7 Part 1 (Lot: 16-14), Decon 7 Part 2 (Lot: 16-17), and booster (Lot: 470572802), mixed at a ratio of 1 part Decon 7 Part 1, 1 part Decon 7 Part 2 and 2% booster (v/v), demonstrated 5.88 Log reduction when tested against *C. difficile* ATCC 43598 (endospores) supplemented with a 3 part organic soil load at a contact time of 10 minutes ± 5 seconds. These lots of test substance did not meet the U.S. EPA Product Performance Guidelines for disinfection of *C. difficile* endospores outlined in EPA Product Performance Test Guidelines, OCSPP 810.2100 or the success criteria detailed in the approved protocol.

Test substance Decon 7 Part 1 (Lot: 16-15), Decon 7 Part 2 (Lot: 16-18), and booster (Lot: 470572802), mixed at a ratio of 1 part Decon 7 Part 1, 1 part Decon 7 Part 2 and 2% booster (v/v), demonstrated 6.24 Log reduction when tested against *C. difficile* ATCC 43598 (endospores) supplemented with a 3 part organic soil load at a contact time of 10 minutes ± 5 seconds. These lots of test substance met the U.S. EPA Product Performance Guidelines for disinfection of *C. difficile* endospores outlined in EPA Product Performance Test Guidelines, OCSPP 810.2100 or the success criteria detailed in the approved protocol.

The study was carried out in compliance with the approved protocol (P1448) unless otherwise stated on page 10 of this report. All experimental controls met the established acceptance criteria.

REFERENCES

- "ASTM International" Standard Quantitative Disk Carrier Test Method for Determining Bactericidal, Virucidal, Fungicidal, Mycobactericidal, and Sporicidal Activities of Chemicals. March 2011. ASTM E2197
- Standard Operating Procedure MB-31-03; Quantitative Disk Carriers Test Method (QCT-2) Modified for Testing Antimicrobial Products Against Spores of *Clostridium difficile* (ATCC 43598) on Inanimate, Hard Non-porous Surfaces.
- EPA Product Performance Test Guidelines, OCSPP 810.2100 Sterilants & Sporicides – Recommendations for Efficacy Testing, 4 SEP 2012.

CERTIFICATE OF ANALYSIS

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

January 14, 2016
Certificate of Analysis Decon 7 Part 1

Decon7 Part 1 contains 2 quaternary ammonium compounds. The active [Alkyl Dimethylbenzeyl Ammonium Chloride] is added the production batch first, then the batch is assayed for Quat. concentration using method BCQCSP-2.11

batch number	% wt. Alkyl Dimethylbenzyl Ammonium Chloride (Active)	LCL	UCL
16-13	3.08	3.04	3.36
16-14	3.06	3.04	3.36
16-15	3.09	3.04	3.36

www.baumscastorine.com

CERTIFICATE OF ANALYSIS

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

January 14, 2016

Certificate of Analysis DF 200 Part 2

. Part 2 is assayed for %wt.H_2O_2 using method BCQCSP – 6.44. Expiration date to all product is **1/14/17.**

batch number	% wt. H_2O_2	LCL	UCL
16-16	7.604	7.6	8.4
16-17	7.586	7.6	8.4
16-18	7.584	7.6	8.4

www.baumscastorine.com

PROTOCOL

Protocol for ASTM E2197 – 11 Standard Quantitative Disk Carrier Test Method
P1448

Test Microorganism(s)
Clostridium Difficile ATCC 43598 (endospores)

Product Identity
Decon 7 Part 1
Lots: 16-13, 16-14, and 16-15
Decon 7 Part 2
Lots: 16-16, 16-17, and 16-18
Booster
Lot: 4705722802

Data Requirement
US EPA 40 CFR Part 158
U.S. EPA OCSPP 810.2100

Study Sponsor
Joe Drake
Decon7 Systems, LLC
7575 E. Redfield Rd., Suite 235
Scottsdale, AZ 85260

Performing Laboratory
Microchem Laboratory
1304 W. Industrial Blvd.
Round Rock, Texas 78681

Protocol Number
P1448

Study Director
Eric Cuellar, B.S.

Date
17FEB2016

PROTOCOL

I. Introduction

This document details the materials and procedure for evaluating the efficacy of disinfectants for claims against *Clostridium difficile* using the ASTM E2197 Standard Quantitative Disk Carrier Test Method (QCT-II) in accordance with Good Laboratory Practice Standards (GLPs) stipulated by 40 CFR 160. Modifications to the official method have been incorporated to account for recommendations outlined in EPA BEAD SOP MB-31-03. 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 specified test parameters.

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

The United States Environmental Protection Agency (USEPA) requires specific antimicrobial claims made for disinfectants for use on hard surfaces and sold in the United States to be supported by relevant test systems (microorganisms) outlined in EPA Product Performance Test Guidelines, OCSPP 810.2100, Sterilants & Sporicides-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.htm

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

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 — **Decon 7 Part 1**
Lot Number(s) — **16-13, 16-14, and 16-15**
Active Ingredient & Concentration — **Certificates of Analysis to be included in final report**
Manufacture Date — **04 JAN 2016**
Expiration Date — **14 JAN 2017**

Test Substance Name — **Decon 7 Part 2**
Lot Number(s) — **16-16, 16-17, and 16-18**
Active Ingredient & Concentration — **Certificates of Analysis to be included in final report**
Manufacture Date — **04 JAN 2016**
Expiration Date — **14 JAN 2017**

Test Substance Name — **Booster**
Lot Number(s) — **4705722802**
Active Ingredient & Concentration — **N/A**

Special Handling Requirements — None

Test substance characterization as to content, stability, etc., (40 CFR, Part 160, and Sub part F [160.105]) is the responsibility of the Study Sponsor. The test substance shall be characterized by the Sponsor prior to the completion of this study.

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.

PROTOCOL

VI. Study Parameters, Incorporated by Reference

Number of Tests Comprising the Study — **3 (1 Test per Test Substance Lot per Test Microorganism, conducted on 3 separate days)**
Carrier Type — **Brushed Stainless Steel Disks (1 cm diameter, 0.7 mm thickness)**
Number of Carriers per Test Substance Lot — **10**
Number of Carriers per Control Substance – **3**
Test Substance Form — **Dilution required (1:1), e.g. 1 part Decon7 Part 1 + 1 part Decon 7 Part 2 + 2 % ± 0.1% (v/v) Booster**
Test Temperature — **20 - 25°C**
Contact Time —**10 minutes ± 5 seconds**
Organic Soil Load — **3-part soil load consisting of bovine serum albumin, yeast extract, and mucin**
Neutralization Broth — **To be noted in final report**

Proposed Experimental Start Date: 23FEB2016
Proposed Experimental Termination Date: 01MAR2016

VII. Test System (Microorganism

Clostridium Difficile ATCC 43598 (endospores)

VIII. Materials

- Purified spore suspension of the test system demonstrating ≥95% spores.
- Sufficient quantity of brushed, magnetized stainless steel disks 1 cm in diameter, approximately 0.7mm thick. A minimum of 13 carriers (10 test and 3 control) are needed per lot of test substance, extra carriers may be prepared to be noted in the datasheet.
- Sufficient volume of test substance.
- Bunsen burner, microbiological incinerator, or micro-torch as appropriate to ensure rapid and complete flame-sterilization of wire inoculating loops and forceps.
- Sufficient quantity of clean, sterile 100 x 15 mm Petri dishes (glass, pyrex, and/or plastic).
- Sufficient number and volume of sterile Petri dishes containing sterile, pre-reduced Brain Heart Infusion Agar with yeast extract supplemented with 0.1% Sodium Taurocholate and 7.0% horse blood.
- Filtration system with sufficient quantity of 0.2 μm Polyethersulfone (PES) membrane filters for enumeration via membrane filtration.
- Sufficient quantity of sterile, wide-mouth Nalgene vials (30 ml) or equivalent for holding inoculated test carriers during test.
- Sufficient volume of phosphate-buffered saline (PBS).
- Sufficient volume of phosphate-buffered saline containing 0.1% (v/v) Tween 80 (PBS-T)
- Sufficient volume of organic soil load constituents
- Micropipettes and a sufficient quantity of appropriately sized sterile micropipette tips.
- Calibrated positive displacement pipette, capable of dispensing 0.01 ml volumes

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- Automatic pipettor (pipetAid or similar) and various sizes of sterile serological pipettes.
- Certified satellite clock.
- Certified digital timer.
- Thermometer.
- Desiccator with Desiccant
- Vacuum Source.
- Vortex mixer.
- Forceps.
- Filter Paper.
- Incubator capable of sustaining temperaturs of 36 ± 1°C
- Chambers capable of sustaining anaerobic conditions during incubation of the test system (microorganism).
- Anaerobic sachets.
- Biological safety cabinet.
- Magnet

IX. Procedure

Preparation of Test Tubes and Test Substance

- Prior to use in the study the Test substance is prepared by combining 1 part Decon 7 part 1 with 1 part Decon 7 part 2. Prepared test substance is supplemented with Booster at a concentration of 2.0 ± 0.1% (v/v) then allowed to dwell for a minimum of 10 minutes.
- Test substance is equilibrated to test temperature for ≥ 10 minutes prior to efficacy testing.
- Test substance is used within 3 hours of preparation.

Preparation of Neutralization Broth

- Before the test begins, the neutralization broth is prepared in bulk and steam sterilized.

Preparation of Test Carriers

- Prior to use in the study, carriers are observed for flaws and flawed carriers are discarded.
- Before the test, clean stainless steel carriers are soaked in a suitable detergent for 1-2 hours.
- Carries are thoroughly rinsed using multiple tap-water rinses followed by a double R/O water rinse.
- Carriers are placed, rounded edge up, in a filter paper lined petri dish. Up to 20 carriers are placed in each dish and carriers are steam sterilized.
- Carriers are aseptically transferred to a sterile petri dish not containing filter paper.
- Carriers are allowed to dry and cool to room temperature prior to inoculation.

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Preparation of Recovery/Plating Medium

- Brain heart infusion agar with yeast extract is prepared according to manufacturer's recipe and supplemented to contain 0.1% Sodium Taurocholate. The agar is steam sterilized and tempered to approximately 49°C prior to additionally supplementing with 7.0% defibrinated horse blood.
- Molten agar is poured into plates and allowed to solidify.
- At least 18 hours prior to use in the study, solidified agar plates are incubated in anaerobic conditions to ensure media is oxygen reduced.

Preparation of Organic "Soil" Load for Test organism

The soil load consists of a mixture of the following solutions prepared in PBS:

- Yeast Extract Stock: 0.5 g of yeast extract is added to 10 ml of PBS, mixed, and passed through a 0.2 μm pore diameter membrane filter.
- Bovine Serum Albumin Stock: 0.5 g of BSA is added to 10 ml of PBS, mixed and passed through a 0.2 μm pore diameter membrane filter.
- Mucin (bovine or porcine) Stock: 0.04 g of mucin is added to 10 ml PBS, mixed and autoclave sterilized.
- These solutions are prepared separately, stored in single use containers, and frozen for up to one year.

Preparation of Test Culture Suspension

- Frozen stock spore suspension is prepared and the spore suspension preparation is documented according to Test Facility Operation 023.4.
- Frozen spore suspension is thawed and vortex mixed to resuspend the spores.
- To obtain a 0.500 ml volume of supplement spore preparation, components are combined as follows:
  - 0.025 ml BSA stock
  - 0.035 ml yeast extract stock
  - 0.100 ml mucin stock
  - 0.340 ml spore suspension
- Different final volumes of supplemented spore suspension may be prepared maintaining the correct ratio of each component.
- The final supplemented spore suspension is vortex mixed prior to use.

Inoculation of Carriers with Supplemented Spore Suspension

- Using a positive displacement pipette, each test carrier is inoculated with 0.010 ml of supplemented spore suspension directly in the center of each carrier, avoiding contact with the carrier. The same pipette tip is used to inoculate all carriers if possible. The inoculum is not spread, but allowed to bead on the center of the carrier.

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- Inoculated carriers are placed in a biological safety cabinet with Petri dish lids off and allowed to dry for 30 ± 5 minutes.
- Carriers are transferred to a desiccator without lids.
- A vacuum is applied at room temperature for 2 hours.
- After drying carriers are observed and only visually dry carriers are used in the test.
- Any carriers in which the inoculum as run over the edge are discarded and not used for efficacy testing.
- Dried carriers may be stored for 24 hours in a desiccator without a vacuum applied, carriers are used within 24 hours of drying.
- Carriers deemed acceptable for use are aseptically transferred to the bottom of sterile nalgene vials or equivalent, using flame-sterilized forceps, with the inoculated side of the carriers facing up.

Exposure of Carriers to Test Substance

- Using a certified timer, 0.050 ml of test substance is deposited over the dried inoculum on each test carrier ensuring complete coverage. Carriers are treated at predetermined, staggered intervals using a new pipette tip for each carrier
- Vials are left uncapped for the duration of the contact time at 20-25°C.
- At the end of the sponsor specified contact time, 10 ml of neutralization buffer is added to each vial and briefly vortex mixed (e.g. 2-3 sec.). The neutralized vial represents the 10^0 dilution.
- Following neutralization of the entire set of carriers (treated and control) each vial is vortex mixed for 30 ± 5 seconds at high speed.
- In the event of incomplete elution, further vortexing may be performed at the discretion of the study director.

Exposure of Carriers to Control Substance

- After treatment of the test carriers and in line with the staggered intervals using a certified timer, 0.050 ml of PBS-T is deposited over the dried inoculum on each carrier ensuring complete coverage. Carriers are treated using a new pipette tip for each carrier.
- Vials are left uncapped for the duration of the contact time at 20-25°C.
- At the end of the sponsor specified contact time, 10 ml of neutralization buffer is added to each vial and briefly vortex mixed (e.g. 2-3 sec.). The neutralized vial represents the 10^0 dilution.
- Following neutralization of the entire set of carriers (treated and control) each vial is vortex mixed for 30 ± 5 seconds at high speed.
- In the event of incomplete elution, further vortexing may be performed at the discretion of the study director.

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Enumeration of Neutralized Test Carriers

- A filtration system is set up with 0.2 μm Polyethersulfone (PES) membrane filter for enumeration via membrane filtration.
- Prior to filtration, each membrane is pre-wet with approximately 10 ml of sterile PBS.
- The entire contents of nalgene tubes containing treated carriers are filtered, taking care to keep the carrier in the nalgene vial using a magnet, or other means as appropriate.
- Each vial is washed with approximately 20 ml of sterile PBS and briefly vortex mixed. The wash liquid is then poured into the same membrane filter. This step is repeated 3 more times for each test carrier for a total of 4 washes per vial.
- After all washes are complete the vacuum is applied to the filter
- With the vacuum on, the membrane filter and funnel are washed once with approximately 40 ml of sterile PBS after the last wash has been filtered.
- The membrane filter is aseptically transferred to a pre-reduced growth supporting agar.
- Plates with membrane filters are placed under anaerobic conditions within 50 ± 10 minutes of removing plates from reducing conditions.

Enumeration of Neutralized Control Carriers

- This step is performed after treated carriers have been filtered
- A filtration system is set up with 0.2 μm Polyethersulfone (PES) membrane filter for enumeration via membrane filtration.
- Prior to filtration, each membrane is pre-wet with approximately 10 ml of sterile PBS.
- For control carriers, serial 1:10 (1 ml into 9 ml) dilutions are conducted in PBS-T. The entire contents of tubes representing the appropriate dilutions are filtered.
- Each vial is washed with approximately 10 ml of sterile PBS and briefly vortex mixed. The wash liquid is then poured into the same membrane filter. Only a single wash is required.
- After the wash is complete the vaccum is applied to the filter.
- With the vacuum on, the membrane filter and funnel are washed once with approximately 20 ml of sterile PBS after the last wash has been filtered.
- The membrane filter is aseptically transferred to a pre-reduced growth supporting agar.
- Plates with membrane filters are placed under anaerobic conditions within 50 ± 10 minutes of removing plates from reducing conditions.

Neutralization Control

- A 0.050 ml aliquot of test substance is added directly to a sterile vial containing 10 ml of the neutralizaer used in the test and represents the "Neutralization Test."
- A 0.050 ml aliquot of sterile PBS is added directly to a vial containing 10 ml of the neutralizer used in the test and represents the "Neutralization Control."

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- Supplemented spore suspension is diluted in sterile PBS-T to a concentration of $\leq 1x10^3$ CFU/ml. 0.100 ml of the appropriate dilution is added directly to each vial and vortex mixed.
- The entire contents of each vial is passed through a separate 0.2 μm filter.
- Each vial is washed with approximately 20.0 ml sterile PBS and the wash volume is passed through the same membrane filter. This step s repeated three additional times for a total of 4 washes per vial.
- The membrane filter and funnel are washed once with approximately 40 ml volume of sterile PBS after the last wash is filtered.
- The membrane filter is aseptically transferred to a pre-reduced growth supporting agar.
- Plates with membrane filters are placed under anaerobic conditions within 50 ± 10 minutes of removing plates from reducing conditions.
- Test system growth on the filters representing the "Neutralization Test" are directly compared to test system growth on the filters representing the "Neutralization Control."

Neutralization Broth Sterility Control

- An aliquot of neutralization broth is struck to recovery media and incubated alongside enumeration plates to verify sterility at the time of test.

"Soil" Sterility Control

- An aliquot of test soil is struck to recovery medium and incubated alongside enumeration plates to verify sterility at the time of test if applicable.

Test Microorganism Purity Control

- A loopful of the supplemented spore suspension used in this study is subcultured to recovery medium and incubated alongside enumeration plates to morphologically confirm the presence of a pure culture.

Media Sterility Control

- An aliquot of PBS is struck to sterile growth medium and incubated alongside enumeration plates to verify sterility at the time of test.
- An aliquot of PBS-T is struck 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 along side test materials to verify sterility at the time of test.

Incubation of Recovery/Enumeration and Control Plates

- Control Plates are incubated anaerobically for 48 ± 4 hours at 36 ± 1°C
- Treated plates are incubated anaerobically for 72 ± 4 hours at 36 ± 1°C

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- If there are zero or few colonies after the initial 72 ± 4 hour incubation on treated carriers, plates are incubated for an additional 48 ± 4 hours under anaerobic conditions at 36 ± 1°C

Calculations

- Colony counts of >200 CFU/filter are recorded as TNTC. For treated carrier calculations, values of 200 will be substituted for TNTC and are scaled up.

$$\frac{(\text{Average CFU for } 10^{-X}) + (\text{Average CFU for } 10^{-Y}) + (\text{Average CFU for } 10^{-Z})}{10^{-X} + 10^{-Y} + 10^{-Z}} = \text{CFU/ml}$$

[(CFU/ml) X 10] = CFU/Carrier

Note: Other dilutions may be plated in the event that a lower viable concentration is expected.
Log_{10} density per carrier (treated or control) = $\log_{10}$(CFU/Carrier)

Log_{10} reduction = mean $\log_{10}$ control carriers – mean $\log_{10}$ treated carriers

Note: if no spores are recovered from any treated carriers, log reduction is reported as greater than log10 density of control carriers.

X. Success Criteria

- The experimental success (controls) criteria follow:
  - Control carriers must demonstrate a mean log density of at least 6.0 (corresponding to $1x10^6$ CFU/ Carrier) and not above 7.0 (corresponding to a mean log density of $1x10^7$ CFU/Carrier.)
  - The neutralizer sterility control is negative for growth.
  - The carrier sterility control is negative for growth.
  - The viability growth control is positive for growth.
  - The Test system purity control demonstrates a pure spore suspension.
  - The neutralization control inoculum demonstrates ≤100 CFU.
  - The "soil" sterility control is negative for growth.
  - The media sterility controls are negative for growth.
- The EPA performance criterion for disinfection follows:
  - A minimum of 6 $\log_{10}$ reduction in viable spores is observed under the conditions tested.
- Retesting guidance for disinfection follows:
  - When a test fails and the $\log_{10}$ density of the test carriers is below 6.0, no retesting is necessary.
  - When a test passes and the $\log_{10}$ density of the test carriers is above 7.0, no retesting is necessary.
  - When a test fails and the $\log_{10}$ density of the test carriers is above 7.0, retesting may be conducted.

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XI. 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.

XII. Data and Sample Retention

- The study report and corresponding data sheets will be held in the archives of Microchem Laboratory for at least 2 years after the date of the final report and then may be destroyed. If the study is used by the Study Sponsor in support of a label claim, documentation may be returned to the Study Sponsor for archiving at Study Sponsor's expense.
- 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 may be destroyed >30 days after study completion.

XIII. 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.

XIV. References

- "ASTM, International." Standard Quantitatvie Disk Carrier Test Method for Deterimining Bactericidal, Virucidal, Fungicidal, Mycobactericidal, and Sporicidal Activities of Chemicals. March 2011. ASTM E2197
- Standard Operating Procedure MB-31-03; Quantitative Disk Carriers Test Method (QCT-2) Modified for Testing Antimicrobial Products Against Spores of *Clostridium difficile* (ATCC 43598) on Inanimate, Hard Non-porous Surfaces.
- EPA Product Performance Test Guidelines, OCSPP 810.2100 Sterilants & Sporicides – Recommendations for Efficacy Testing, 4 SEP 2012.

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XV. 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

Joe Drake, Study Sponsor, Decon7 Systems, LLC — Date: 2/22/2016

Eric Cuellar, Study Director, Microchem Laboratory, LLC — Date: 23 FEB 2016

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