STUDY TITLE
Testing Disinfectants Under Simulated Industrial Laundry Conditions

Study Identification Number
GLP1362

Protocol Number
P1428

Product Identity
Test Substance: Decon7 Part 1 (Lots: 16-13, 16-14, and 16-15), Decon7 Part 2 (Lots: 16-16, 16-17, and 16-18), and Booster (Lot: 470572802)

Test Microorganism(s)
Staphylococcus aureus ATCC 6538
Klebsiella pneumoniae ATCC 4352

Data Requirements
U.S. EPA 40 CFR Part 158
U.S. EPA OCSPP 810.2400

Author
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Study Director

Study Completion Date
26 APR 2016

Testing Facility
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Study Sponsor
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7575 E. Redfield Rd., Suite 235
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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:

- Records concerning test substance characteristics (i.e. composition, purity, stability, strength, solubility) are maintained by the Study Sponsor. The Study Sponsor conducted test substance characterization as to identity, strength, purity, solubility and composition, as applicable, according to 40 CFR Part 160, Subpart F [160.105] prior to its use in the study.

Study Director

Company: Microchem Laboratory

Name: Elizabeth Richard, B.S.

Title: Study Director

Signature: _____ Date: 26 APR 2016

Study Sponsor

Company: Decon7 Systems, LLC

Name: Joe Drake

Title: Study Sponsor

Signature: _____ Date: _____

Submitter

Company:

Name:

Title:

Signature: _____ Date: _____

QUALITY ASSURANCE STATEMENT

Study Title: Testing Disinfectants Under Simulated Industrial Laundry Conditions

Study ID#: GLP1362

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	02 FEB 2016	04 FEB 2016	04 FEB 2016
Draft Report	29 MAR 2016	01 APR 2016	08 APR 2016
Final Report	26 APR 2016	26 APR 2016	26 APR 2016

Quality Assurance Unit

Signature: _____ Date: 26 APR 2016

Name: Nicholas Broughton, B.S.

Title: Quality Assurance Specialist

PERSONNEL INVOLVED IN THE STUDY

Study Director
Name: Elizabeth Richard, B.S.
Company: Microchem Laboratory
Title: Study Director

Scientific Director
Name: Jason Williams, B.S.
Company: Microchem Laboratory
Title: Scientific Director

Assisting Personnel
Name: Blake Rolland, B.S.
Company: Microchem Laboratory
Title: Technician

Name: Donald DeClue, B.S.
Company: Microchem Laboratory
Title: Technician

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FINAL STUDY REPORT SUMMARY

Study Title
Testing Disinfectants Under Simulated Industrial Laundry Conditions

Study Identification Number
GLP1362

Protocol Number
P1428

Test Microorganism(s)
Staphylococcus aureus ATCC 6538
Klebsiella pneumoniae ATCC 4352

Study Sponsor
Joe Drake
Decon7 Systems, LLC
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Scottsdale, AZ 85260

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

Study Director
Elizabeth Richard, B.S.

Study Completion Date
26 APR 2016

Study Objective
To determine, using the ASTM E2274 Method for Evaluation of Laundry Disinfectants, the antimicrobial efficacy of Decon7 Part 1 (Lots: 16-13, 16-14, and 16-15), Decon7 Part 2 (Lots: 16-16, 16-17, and 16-18), and the Booster (Lot: 470572802) against *Staphylococcus aureus* ATCC 6538 and *Klebsiella pneumoniae* ATCC 4352 supplemented with 5% ± 0.1% (v/v) fetal bovine serum at a contact time of ≤10 minutes.

Study Conclusion in Brief
Decon7 Part 1 (Lots: 16-13, 16-14, and 16-15), Decon7 Part 2 (Lots: 16-16, 16-17, and 16-18), and the Booster (Lot: 470572802), when combined for this assay, met the U.S. EPA OCSPP 810.2400 for disinfection success criteria when tested against *Staphylococcus aureus* ATCC 6538 and *Klebsiella pneumoniae* ATCC 4352.

FINAL STUDY REPORT

Important Dates

Study Initiation Date: 02 FEB 2016
Experimental Start Date/Time: 02 FEB 2016 / 1215
Experimental End Date/Time: 25 FEB 2016 / 1404

Test Substance Information

Name: Decon7 Part 1, Decon7 Part 2, and Booster

Lots: Decon7 Part 1 (Lots: 16-13, 16-14, and 16-15)
Active Ingredient (Concentration): Alkyl Dimethylbenzyl Ammonium Chloride (3.08, 3.06, and 3.09 % wt. respectively)
Date Received: 20 JAN 2016
Expiration Date: 14 JAN 2017

Decon7 Part 2 (Lots: 16-16, 16-17, and 16-18)
Active Ingredient (Concentration): Hydrogen Peroxide (7.604, 7.586, and 7.584 % wt. respectively)
Date Received: 20 JAN 2016
Expiration Date: 14 JAN 2017

Booster (Lot: 470572802)
Inert Ingredient

Storage Conditions:	Ambient Temperature under Fluorescent Lighting
Form (Dilution):	Liquid substance requiring dilution (1:1:42 plus Booster added at 2% v/v of Decon7 Part 1 and Decon7 Part 2)
Diluent Type:	200 ± 10 PPM AOAC Synthetic Hard Water Solution
Vol. of Test Substance:	75.0 ± 0.1ml of diluted test substance per test chamber
Control Substance:	200 ± 10 PPM AOAC Synthetic Hard Water Solution supplemented with 0.5% (v/v) Triton X-100
Vol. of Control Substance:	75.0 ± 0.1ml of control substance per control chamber
Fabric to Wash Water Ratio:	1:5 (e.g. 15.0 ± 0.1 g fabric to 75.0 ± 0.1 ml dilute test substance)

FINAL STUDY REPORT (cont.)

Test Parameters

Microorganism(s):	*Staphylococcus aureus* ATCC 6538 *Klebsiella pneumoniae* ATCC 4352
Subculture Number(s):	3, 4, and 5
Culture Manipulation:	Test culture was diluted in Phosphate Buffered Saline, dilution reported in tables, prior to addition of organic soil load
Number of Test Carriers:	9 carriers per lot (3 spindles, 3 carriers/spindle)
Number of Control Carriers:	3 carriers per lot (1 spindle, 3 carriers/spindle)
Spindle Parameters:	Approximately 2 inch wide strips weighing 15 ± 0.1g wrapped 12 times around metal spindle
Carrier Type:	1" x 1.5" scoured cotton fabric carriers
Contact Time:	≤10 minutes
Test Temperature:	Ambient, recorded at time of test
Agitation Parameters:	360° vertical orbit of 4-8 inches at 45-60 RPM
Neutralization Broth:	Dey Engley Broth supplemented with 0.1% Catalase
Organic Soil Load:	5% ± 0.1% (v/v) Fetal Bovine Serum (FBS)
Carrier Dry Time:	≤30 minutes
Carrier Dry Temperature:	36 ± 1°C
Incubation Temperature:	36 ± 1°C
Incubation Time:	48 to 54 hours

Test Method

The test was conducted according to the attached protocol unless noted on pages 10-11.

PROTOCOL CHANGES

Protocol Amendment(s)

Amendment #1:

The signed protocol (P1428) is hereby amended to include the following change in section VI:

"Contact Time – **≤9.5 Minutes** "

is amended to

"Contact Time – **≤10 Minutes** "

"Carriers are harvested at intervals before 9.5 minutes (for example: the first carrier at 9 minutes and 10 seconds, the second carrier at 9 minutes and 20 seconds, and the third carrier at 9 minutes and 30 seconds). All harvest times are recorded."

is amended to

"Carriers are harvested at intervals before 10 minutes (for example: the first carrier at 9 minutes and 40 seconds, the second carrier at 9 minutes and 50 seconds, and the third carrier at 10 minutes). All harvest times are recorded."

Amendment #2:

The signed protocol (P1428) is hereby amended to include the following change in the entire document:

The lot number for Decon7 Booster is amended from 4705722802 to 470572802.

Protocol Deviation(s)

Deviation #1:

The temperature of incubator A1211 was recorded out the range stated in protocol P1428 on the afternoon of 23FEB2016 (37.3°C) and the morning of 25FEB2016 (37.2°C). At both deviation occurences, GLP1362, specifically Lots: 16-15, 16-18, and 470572802 for *S. aureus* ATCC 6538, was incubating the test materials. The elevated temperature was thought not to have effected the outcome of the study due to the passing result despite the optimal conditions for growth of the microorganism.

PROTOCOL CHANGES (cont.)

Deviation #2:

On 02 FEB 2016, a single carrier in the first spindle of Lots: 16-13, 16-16, and 470572802 against *K. pneumoniae* ATCC 4352 was neutralized two seconds after the contact time, ≤9.5 minutes. The increased contact time did not affect the outcome of the study due to the insignificance of two seconds compared to the total contact time, 9.5 minutes.

Deviation #3:

On 01 FEB 2016, *S. aureus* ATCC 6538 and *K. pneumoniae* ATCC 4352 were transferred outside of the protocol stated transfer range, 24 ± 6 hours. This deviation did not affect the outcome of the study due to the robust growth of the subsequent transfers and the inoculum concentration being within the ranges specified in the protocol.

Deviation #4:

On 04 FEB 2016, the neutralization verification for Lots: 16-13, 16-16, and 470572802 against *S. aureus* ATCC 6538 was evaluated and the resulting counts for the colony forming units were outside the range stated in the protocol, 10-100 CFU. The average count recorded was 289.5 CFU. Therefore, the neutralization verification for Lots: 16-13, 16-16, and 470572802 against *S. aureus* ATCC 6538 was repeated on 08 FEB 2016 with a valid result and an average count of 20.5.

Deviation #5:

Fabric lots, STF09DEC2015A and STF17FEB2016A prepared on 09 DEC 2015 and 17 FEB 2016 respectively, were not prepared according to the protocol. The fabric was prepared in smaller batches with the ratios of Triton X-100 and sodium carbonate maintained. This deviation did not affect the outcome of the study because the fabric to rinsing liquid ratio remained consistent with the ratio outlined in the protocol.

CONTROLS

Enumeration of Count Control Carriers
Following the conclusion of the dry time, three dried inoculated carriers were assayed immediately prior to conducting the test. Each carrier was aseptically transferred to individual sterile subculture/neutralization test tubes. These test tubes were vortex mixed for 120 ± 5 seconds to elute the microorganisms then individually enumerated using standard dilution and plating techniques.

Enumeration of Control Carriers and Wash Water
Three inoculated carriers were placed in the control substance and agitated in the same manner as the carriers treated with test substance per the attached protocol. Agitation ceased prior to the contact time to allow for harvesting of three carriers and three aliquots of wash water. Tubes with control carriers were vortex mixed for 120 ± 5 seconds to elute the microorganisms then individually enumerated using standard dilution and plating techniques. Tubes containing aliquots of wash water were thoroughly vortex mixed then individually enumerated using standard dilution and plating techniques.

Carrier Sterility Control
An uninoculated carrier was transferred to a sterile test tube containing the subculture/ neutralization broth to confirm carrier sterility.

Viability Control
One inoculated test carrier was placed in an individual subculture/neutralization broth tube and incubated alongside the test to confirm test system viability.

Media Sterility Controls
A tube containing only subculture/neutralization broth was incubated alongside the test to confirm subculture/neutralization broth sterility.
A plate containing growth medium was incubated alongside the test to confirm plating media sterility.
Plates containing an aliquot of soil, PBS, culture dilution media, and 200 ± 10 PPM AOAC Hard Water were plated using growth media and incubated alongside the test to confirm sterility.

Neutralization Control
Three sterile uninoculated carriers were placed in the test substance and agitated in the same manner as the treatment of test carriers. Agitation ceased prior to the contact time to allow for harvesting all three carriers and all three aliquots of wash water into sterile subculture/neutralization broth test tube. After transfer, the test tube was inoculated with between 10 and 100 CFU of test microorganism (obtained by serial dilution) and incubated along with the other test tubes. An additional subculture/neutralization broth tube was inoculated and incubated alongside the test as a positive comparison. The inoculum was plated in duplicate to verify the number of CFU added and incubated alongside the remainder of the test.

CONTROLS (cont.)

Test Microorganism Purity Control

A loopful of each test microorganism used in this study was subcultured for isolation to a petri dish containing appropriate growth agar medium and incubated alongside enumeration plates to morphologically confirm the presence of a pure culture at the time of test.

STUDY ACCEPTANCE CRITERIA

The experimental success (controls) criteria follow:

- All media sterility controls must be negative for growth.
- Carrier sterility control must be negative for growth.
- Carrier viability control is positive for growth.
- The media viability control must be positive for growth.
- All test microorganisms must demonstrate culture purity.
- Neutralization tubes, test and controls, demonstrate turbidity (growth) of test microorganism and the inoculum enumeration yields ≤100 CFU.
- An average of at least 1.0×10^4 CFU/carrier must be recovered from the inoculated washed control fabric carriers and an average of at least 1.0×10^4 CFU/ml must be recovered from the inoculated wash water treated with the control solution.

CALCULATIONS AND STATISTICAL ANALYSIS

The following are calculations to be used in the study. Calculation variables may be adjusted based on volumes and dilutions used.

(Average CFU on plated plated per ml) X 10^x = CFU/ml

Where "x" represents the dilution factor

Dilution used for wash water calculation:

$$\frac{\text{20ml of neutralizer broth}}{\text{0.5 ml harvested from wash water}} = 40$$

(Average CFU on plated plated per carrier) X 10^x = CFU/carrier

Where "x" represents the dilution factor

Log_{10} Density = Log_{10} (CFU/Carrier)

Neutralization Verification Inoculum = (CFU on plate 1 + CFU on plate 2)/2

$$\frac{\text{(Concentration in ppm of } CaCO_3\text{) x (volume in ml of EDTA used)}}{\text{(Volume in ml of hard water used for titration)}} = \text{Concentration of hard water in ppm}$$

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 a minimum of 2 years after the date of the final report. After this time, the Sponsor will be contacted to determine the final disposition. Study report and corresponding data returned to the Study Sponsor will be returned at the Sponsor's expense.

The original data includes, but is not limited to the following:

- 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 Sponsor's request and expense within 30 days of study completion. If the Study Sponsor does not request the return of the sample, it will be destroyed 30 days after study completion.

RESULTS

Table 1
The following were the enumeration results for the inoculum for Decon7 Part 1, Part 2, and Booster (Lots: 16-13, 16-16, 470572802) against *S. aureus* ATCC 6538 tested on 02 FEB 2016.

Test Microorganism	Test Substance	Dilution	Mean CFU/ml	Mean Log_{10} Density
S. aureus ATCC 6538	Lots: 16-13, 16-16, 470572802	Inoculum 1:60	6.55E+08	8.82

Table 2
The following were the enumeration results for the inoculum for Decon7 Part 1, Part 2, and Booster (Lots: 16-14, 16-17, 470572802 and Lots: 16-13, 16-16, 470572802) against *S. aureus* ATCC 6538 tested on 08 FEB 2016.

Test Microorganism	Test Substance	Dilution	Mean CFU/ml	Mean Log_{10} Density
S. aureus ATCC 6538	Lots: 16-14, 16-17, 470572802	Inoculum 1:60	2.40E+08	8.38

**Neutralization validation repeated for Decon 7 (Lots: 16-13, 16-16, 470572802) against S. aureus ATCC 6538 used this inoculum.*

Table 3
The following were the enumeration results for the inoculum for Decon7 Part 1, Part 2, and Booster (Lots: 16-15, 16-18, 470572802) against *S. aureus* ATCC 6538 tested on 22 FEB 2016.

Test Microorganism	Test Substance	Dilution	Mean CFU/ml	Mean Log_{10} Density
S. aureus ATCC 6538	Lots: 16-15, 16-18, 470572802	Inoculum 1:60	2.30E+08	8.36

RESULTS (cont.)

Table 4
The following were the enumeration results for the Carrier Count Control for Decon7 Part 1, Part 2, and Booster (Lots: 16-13, 16-16, 470572802) against *S. aureus* ATCC 6538 tested on 02 FEB 2016.

Test Microorganism	Test Substance	Control	CFU/Carrier	Mean CFU/Carrier	Mean Log_{10} Density
S. aureus ATCC 6538	Lots: 16-13, 16-16, 470572802	Carrier Count Control	1.38E+07	1.30E+07	7.11
			1.27E+07		
			1.25E+07		

Table 5
The following were the enumeration results for the Control Treated Carriers for Decon7 Part 1, Part 2, and Booster (Lots: 16-13, 16-16, 470572802) against *S. aureus* ATCC 6538 tested on 02 FEB 2016.

Test Microorganism	Test Substance	Control	CFU/Carrier	Mean CFU/Carrier	Mean Log_{10} Density
S. aureus ATCC 6538	Lots: 16-13, 16-16, 470572802	Carriers	6.70E+06	8.00E+06	6.90
			9.70E+06		
			7.60E+06		

Table 6
The following were the enumeration results for the Control Wash Water for Decon7 Part 1, Part 2, and Booster (Lots: 16-13, 16-16, 470572802) against *S. aureus* ATCC 6538 tested on 02 FEB 2016.

Test Microorganism	Test Substance	Control	CFU/ml	Mean CFU/ml	Mean Log_{10} Density
S. aureus ATCC 6538	Lots: 16-13, 16-16, 470572802	Wash Water	2.32E+04	1.96E+04	4.29
			1.92E+04		
			1.64E+04		

RESULTS (cont.)

Table 7
The following were the enumeration results for the Carrier Count Control for Decon7 Part 1, Part 2, and Booster (Lots: 16-14, 16-17, 470572802) against *S. aureus* ATCC 6538 tested on 08 FEB 2016.

Test Microorganism	Test Substance	Control	CFU/Carrier	Mean CFU/Carrier	Mean Log_{10} Density
S. aureus ATCC 6538	Lots: 16-14, 16-17, 470572802	Carrier Count Control	6.10E+06	5.77E+06	6.76
			6.10E+06		
			5.10E+06		

Table 8
The following were the enumeration results for the Control Treated Carriers for Decon7 Part 1, Part 2, and Booster (Lots: 16-14, 16-17, 470572802) against *S. aureus* ATCC 6538 tested on 08 FEB 2016.

Test Microorganism	Test Substance	Control	CFU/Carrier	Mean CFU/Carrier	Mean Log_{10} Density
S. aureus ATCC 6538	Lots: 16-14, 16-17, 470572802	Carriers	2.90E+06	3.06E+06	6.49
			2.79E+06		
			3.48E+06		

Table 9
The following were the enumeration results for the Control Wash Water for Decon7 Part 1, Part 2, and Booster (Lots: 16-14, 16-17, 470572802) against *S. aureus* ATCC 6538 tested on 08 FEB 2016.

Test Microorganism	Test Substance	Control	CFU/ml	Mean CFU/ml	Mean Log_{10} Density
S. aureus ATCC 6538	Lots: 16-14, 16-17, 470572802	Wash Water	1.48E+04	1.33E+04	4.12
			1.46E+04		
			1.04E+04		

RESULTS (cont.)

Table 10
The following were the enumeration results for the Carrier Count Control for Decon7 Part 1, Part 2, and Booster (Lots: 16-15, 16-18, 470572802) against *S. aureus* ATCC 6538 tested on 22 FEB 2016.

Test Microorganism	Test Substance	Control	CFU/Carrier	Mean CFU/Carrier	Mean Log_{10} Density
S. aureus ATCC 6538	Lots: 16-15, 16-18, 470572802	Carrier Count Control	5.50E+06	5.67E+06	6.75
			6.70E+06		
			4.80E+06		

Table 11
The following were the enumeration results for the Control Treated Carriers for Decon7 Part 1, Part 2, and Booster (Lots: 16-15, 16-18, 470572802) against *S. aureus* ATCC 6538 tested on 22 FEB 2016.

Test Microorganism	Test Substance	Control	CFU/Carrier	Mean CFU/Carrier	Mean Log_{10} Density
S. aureus ATCC 6538	Lots: 16-15, 16-18, 470572802	Carriers	2.99E+06	2.52E+06	6.40
			3.41E+06		
			1.15E+06		

Table 12
The following were the enumeration results for the Control Wash Water for Decon7 Part 1, Part 2, and Booster (Lots: 16-15, 16-18, 470572802) against *S. aureus* ATCC 6538 tested on 22 FEB 2016.

Test Microorganism	Test Substance	Control	CFU/ml	Mean CFU/ml	Mean Log_{10} Density
S. aureus ATCC 6538	Lots: 16-15, 16-18, 470572802	Wash Water	2.38E+04	2.35E+04	4.37
			2.30E+04		
			2.38E+04		

RESULTS (cont.)

Table 13

The following were the test results for Decon7 Part 1, Part 2, and Booster (Lots: 16-13, 16-16, 470572802) when tested against *S. aureus* ATCC 6538 at a contact time not to exceed 9.5 minutes in the presence of 5% ± 0.1% (v/v) fetal bovine serum (FBS) artificial soil. Agitation parameters were 360° vertical orbit of 4-8 inches at 45-60 RPM, specifically verified at 57 RPM. The pH of Decon7 Part 1, Part 2, and Booster combined without hard water was 10.16, the final prepared test substance had a pH of 9.76. Test was conducted on 02 FEB 2016.

Test Microorganism	Test Substance	Treatment Time	Contact Temperature	Contents of Test Tube	Number of Test Tubes Analyzed	Number of Positive Neutralizer Test Tubes	Number of Confirmed Positive Neutralizer Test Tubes
S. aureus ATCC 6538	Lots: 16-13, 16-16, 470572802	≤9.5 minutes	25.9°C (Room temp at start of contact)	Carriers	9	0	0
				Wash Water	9	0	0

Table 14

The following were the test results for Decon7 Part 1, Part 2, and Booster (Lots: 16-14, 16-17, 470572802) when tested against *S. aureus* ATCC 6538 at a contact time not to exceed 9.5 minutes in the presence of 5% ± 0.1% (v/v) fetal bovine serum (FBS) artificial soil. Agitation parameters were 360° vertical orbit of 4-8 inches at 45-60 RPM, specifically verified at 56 RPM. The pH of Decon7 Part 1, Part 2, and Booster combined without hard water was 10.08, the final prepared test substance had a pH of 9.84. Test was conducted on 08 FEB 2016.

Test Microorganism	Test Substance	Treatment Time	Contact Temperature	Contents of Test Tube	Number of Test Tubes Analyzed	Number of Positive Neutralizer Test Tubes	Number of Confirmed Positive Neutralizer Test Tubes
S. aureus ATCC 6538	Lots: 16-14, 16-17, 470572802	≤9.5 minutes	23.8°C (Room temp at start of contact)	Carriers	9	1*	0
				Wash Water	9	0	0

**A single carrier, Carrier number 5, demonstrated growth after incubation. An aliquot of neutralizer broth was struck to growth agar for isolation. The resulting colony morphology was not typical of the target microorganism.*

RESULTS (cont.)

Table 15

The following were the test results for Decon7 Part 1, Part 2, and Booster (Lots: 16-15, 16-18, 470572802) when tested against *S. aureus* ATCC 6538 at a contact time not to exceed 10 minutes in the presence of 5% ± 0.1% (v/v) fetal bovine serum (FBS) artificial soil. Agitation parameters were 360° vertical orbit of 4-8 inches at 45-60 RPM, specifically verified at 54 RPM. The pH of Decon7 Part 1, Part 2, and Booster combined without hard water was 10.13, the final prepared test substance had a pH of 9.82. Test was conducted on 22 FEB 2016.

Test Microorganism	Test Substance	Treatment Time	Contact Temperature	Contents of Test Tube	Number of Test Tubes Analyzed	Number of Positive Neutralizer Test Tubes	Number of Confirmed Positive Neutralizer Test Tubes
S. aureus ATCC 6538	Lots: 16-15, 16-18, 470572802	≤10 minutes	24.0°C (Room temp at start of contact)	Carriers	9	0	0
				Wash Water	9	0	0

Table 16

The following were the neutralization results for Decon7 Part 1, Part 2, and Booster (Lots: 16-13, 16-16, 470572802) against *S. aureus* ATCC 6538 performed on 08 FEB 2016. Neutralization results were in compliance with the aforementioned study acceptance criteria. The parallel neutralization broth control tube demonstrated positive growth indicative of the target microorganism.

Test Microorganism	Test Substance	Average Inoculum Concentration	Neutralization Verification Result
S. aureus ATCC 6538	Lots: 16-13, 16-16, 470572802	20.5 CFU	Positive Growth, Valid

**The neutralization verification was repeated for Lots: 16-13, 16-16, 470572802 due to an invalid result. The resulting repeated neutralization verification result is valid.*

RESULTS (cont.)

Table 17
The following were the repeated neutralization results for Decon7 Part 1, Part 2, and Booster (Lots: 16-14, 16-17, 470572802) against *S. aureus* ATCC 6538 performed on 08 FEB 2016. Neutralization results were in compliance with the aforementioned study acceptance criteria. The parallel neutralization broth control tube demonstrated positive growth indicative of the target microorganism.

Test Microorganism	Test Substance	Average Inoculum Concentration	Neutralization Verification Result
S. aureus ATCC 6538	Lots: 16-14, 16-17, 470572802	30.5 CFU	Positive Growth, Valid

Table 18
The following were the neutralization results for Decon7 Part 1, Part 2, and Booster (Lots: 16-15, 16-18, 470572802) against *S. aureus* ATCC 6538 performed on 22 FEB 2016. Neutralization results were in compliance with the aforementioned study acceptance criteria. The parallel neutralization broth control tube demonstrated positive growth indicative of the target microorganism.

Test Microorganism	Test Substance	Average Inoculum Concentration	Neutralization Verification Result
S. aureus ATCC 6538	Lots: 16-15, 16-18, 470572802	22.5 CFU	Positive Growth, Valid

RESULTS (cont.)

Table 19
The following were the test microorganism culture incubation conditions and durations used for Decon7 Part 1, Part 2, and Booster (Lots: 16-13, 16-16, 470572802) tested on 02 FEB 2016 against *S. aureus* ATCC 6538 .

Test Microorganism	Transfer Date and Time	Incubation Temperature	Test Culture Transfer	Incubation Time
S. aureus ATCC 6538	29 JAN 2016 / 1322	36 ± 1°C	1	20 hours 20 minutes
	30 JAN 2016 / 0942		2	23 hours 44 minutes
	31 JAN 2016 / 0926		3	31 hours 0 minutes
	01 FEB 2016 / 1626		4	21 hours 53 minutes

Table 20
The following were the test microorganism culture incubation conditions and durations used for Decon7 Part 1, Part 2, and Booster (Lots: 16-14, 16-17, 470572802) tested on 08 FEB 2016 against *S. aureus* ATCC 6538.

Test Microorganism	Transfer Date and Time	Incubation Temperature	Test Culture Transfer	Incubation Time
S. aureus ATCC 6538	04 FEB 2016 / 1510	36 ± 1°C	1	22 hours 55 minutes
	05 FEB 2016 / 1405		2	25 hours 52 minutes
	06 FEB 2016 / 1557		3	23 hours 2 minutes
	07 FEB 2016 / 1459		4	22 hours 5 minutes

RESULTS (cont.)

Table 21
The following were the test microorganism culture incubation conditions and durations used for Decon7 Part 1, Part 2, and Booster (Lots: 16-15, 16-18, 470572802) tested on 22 FEB 2016 against *S. aureus* ATCC 6538.

Test Microorganism	Transfer Date and Time	Incubation Temperature	Test Culture Transfer	Incubation Time
S. aureus ATCC 6538	18 FEB 2016 / 1638	36 ± 1°C	1	23 hours 36 minutes
	19 FEB 2016 / 1614		2	24 hours 13 minutes
	20 FEB 2016 / 1627		3	23 hours 22 minutes
	21 FEB 2016 / 1549		4	20 hours 54 minutes

Table 22
The following were the results for sterility, growth, and purity controls conducted during the study on 02 FEB 2016, 08 FEB 2016 and 22 FEB 2016 against *S. aureus* ATCC 6538.

Study Controls	Result
Carrier Sterility Control Tube	No Growth Observed
Viability Control Tube	Growth-Target Microorganism
Neutralization Media Control Tube	No Growth Observed
Growth Media Control Plate	No Growth Observed
Culture Dilution Media Plate	No Growth Observed
Soil Sterility Control Plate	No Growth Observed
PBS Sterility Control Plate	No Growth Observed
AOAC Hard Water Sterility Control Plate	No Growth Observed
Microorganism Purity Plate	Pure-Target Microorganism

RESULTS (cont.)

Table 23

The following were the incubation times and temperature ranges for the test materials incubated for Decon7 Part 1, Part 2, and Booster (Lots: 16-13, 16-16, 470572802) tested on 02 FEB 2016 against *S. aureus* ATCC 6538.

Test Microorganism	Test Materials	Incubation Temperature	Date / Time to Incubator	Incubation Time
S. aureus ATCC 6538	NV Test and Control Tubes, Test Tubes, Carrier Sterility, Media Sterility, Viability Tubes, Enumeration Plates	36 ± 1°C	02 FEB 2016 / 1633	48 hours 14 minutes
	Confirmation Streak Plates		04 FEB 2016 / 1709	19 hours 16 minutes

Table 24

The following were the incubation times and temperature ranges for the test materials incubated for Decon7 Part 1, Part 2, and Booster (Lots: 16-14, 16-17, 470572802) tested on 08 FEB 2016 against *S. aureus* ATCC 6538.

Test Microorganism	Test Materials	Incubation Temperature	Date / Time to Incubator	Incubation Time
S. aureus ATCC 6538	NV Test and Control Tubes, Test Tubes, Carrier Sterility, Media Sterility, Viability Tubes, Enumeration Plates	36 ± 1°C	08 FEB 2016 / 1552	48 hours 29 minutes
	Confirmation Streak Plates		10 FEB 2016 / 1649	18 hours 43 minutes

**Neutralization validation repeated for Decon 7 (lots: 16-13, 16-16, 470572802) against S. aureus ATCC 6538 used these incubation times.*

RESULTS (cont.)

Table 25
The following were the incubation times and temperature ranges for the test materials incubated for Decon7 Part 1, Part 2, and Booster (Lots: 16-15, 16-18, 470572802) tested on 22 FEB 2016 against *S. aureus* ATCC 6538.

Test Microorganism	Test Materials	Incubation Temperature	Date / Time to Incubator	Incubation Time
S. aureus ATCC 6538	NV Test and Control Tubes, Test Tubes, Carrier Sterility, Media Sterility, Viability Tubes, Enumeration Plates	36 ± 1°C	22 FEB 2016 / 1510	48 hours 37 minutes
	Confirmation Streak Plates		24 FEB 2016 / 1612	21 hours 49 minutes

Table 26
The following were the enumeration results for the inoculum for Decon7 Part 1, Part 2, and Booster (Lots: 16-13, 16-16, 470572802) tested on 02 FEB 2016 against *K. pneumoniae* ATCC 4352.

Test Microorganism	Test Substance	Dilution	Mean CFU/ml	Mean Log_{10} Density
K. pneumoniae ATCC 4352	Lots: 16-13, 16-16, 470572802	Inoculum 1:10	8.50E+09	9.93

Table 27
The following were the enumeration results for the inoculum for Decon7 Part 1, Part 2, and Booster (Lots: 16-14, 16-17, 470572802 and Lots: 16-13, 16-16, 470572802) tested on 04 FEB 2016 against *K. pneumoniae* ATCC 4352.

Test Microorganism	Test Substance	Dilution	Mean CFU/ml	Mean Log_{10} Density
K. pneumoniae ATCC 4352	Lots: 16-14, 16-17, 470572802	Inoculum 1:8	4.55E+09	9.66

RESULTS (cont.)

Table 28

The following were the enumeration results for the inoculum for Decon7 Part 1, Part 2, and Booster (Lots: 16-15, 16-18, 470572802) tested on 10 FEB 2016 against *K. pneumoniae* ATCC 4352.

Test Microorganism	Test Substance	Dilution	Mean CFU/ml	Mean Log_{10} Density
K. pneumoniae ATCC 4352	Lots: 16-15, 16-18, 470572802	Inoculum 1:10	4.55E+09	9.66

Table 29

The following were the enumeration results for the Carrier Count Control for Decon7 Part 1, Part 2, and Booster (Lots: 16-13, 16-16, 470572802) tested on 02 FEB 2016 against *K. pneumoniae* ATCC 4352.

Test Microorganism	Test Substance	Control	CFU/Carrier	Mean CFU/Carrier	Mean Log_{10} Density
K. pneumoniae ATCC 4352	Lots: 16-13, 16-16, 470572802	Carrier Count Control	3.70E+07	6.37E+07	7.80
			8.40E+07		
			7.00E+07		

Table 30

The following were the enumeration results for the Control Treated Carriers for Decon7 Part 1, Part 2, and Booster (Lots: 16-13, 16-16, 470572802) tested on 02 FEB 2016 against *K. pneumoniae* ATCC 4352.

Test Microorganism	Test Substance	Control	CFU/Carrier	Mean CFU/Carrier	Mean Log_{10} Density
K. pneumoniae ATCC 4352	Lots: 16-13, 16-16, 470572802	Carriers	6.30E+05	1.01E+06	6.00
			8.30E+05		
			1.56E+06		

RESULTS (cont.)

Table 31
The following were the enumeration results for the Control Wash Water for Decon7 Part 1, Part 2, and Booster (Lots: 16-13, 16-16, 470572802) tested on 02 FEB 2016 against *K. pneumoniae* ATCC 4352.

Test Microorganism	Test Substance	Control	CFU/ml	Mean CFU/ml	Mean Log_{10} Density
K. pneumoniae ATCC 4352	Lots: 16-13, 16-16, 470572802	Wash Water	2.00E+05	1.80E+05	5.26
			2.10E+05		
			1.30E+05		

Table 32
The following were the enumeration results for the Carrier Count Control for Decon7 Part 1, Part 2, and Booster (Lots: 16-14, 16-17, 470572802) tested on 04 FEB 2016 against *K. pneumoniae* ATCC 4352.

Test Microorganism	Test Substance	Control	CFU/Carrier	Mean CFU/Carrier	Mean Log_{10} Density
K. pneumoniae ATCC 4352	Lots: 16-14, 16-17, 470572802	Carrier Count Control	1.32E+07	1.17E+07	7.07
			1.15E+07		
			1.04E+07		

Table 33
The following were the enumeration results for the Control Treated Carriers for Decon7 Part 1, Part 2, and Booster (Lots: 16-14, 16-17, 470572802) tested on 04 FEB 2016 against *K. pneumoniae* ATCC 4352.

Test Microorganism	Test Substance	Control	CFU/Carrier	Mean CFU/Carrier	Mean Log_{10} Density
K. pneumoniae ATCC 4352	Lots: 16-14, 16-17, 470572802	Carriers	2.51E+06	2.20E+06	6.34
			1.99E+06		
			2.10E+06		

RESULTS (cont.)

Table 34
The following were the enumeration results for the Control Wash Water for Decon7 Part 1, Part 2, and Booster (Lots: 16-14, 16-17, 470572802) tested on 04 FEB 2016 against *K. pneumoniae* ATCC 4352.

Test Microorganism	Test Substance	Control	CFU/ml	Mean CFU/ml	Mean Log_{10} Density
K. pneumoniae ATCC 4352	Lots: 16-14, 16-17, 470572802	Wash Water	2.16E+05	2.21E+05	5.35
			2.42E+05		
			2.06E+05		

Table 35
The following were the enumeration results for the Carrier Count Control for Decon7 Part 1, Part 2, and Booster (Lots: 16-15, 16-18, 470572802) tested on 10 FEB 2016 against *K. pneumoniae* ATCC 4352.

Test Microorganism	Test Substance	Control	CFU/Carrier	Mean CFU/Carrier	Mean Log_{10} Density
K. pneumoniae ATCC 4352	Lots: 16-15, 16-18, 470572802	Carrier Count Control	2.97E+07	2.79E+07	7.45
			2.59E+07		
			2.82E+07		

Table 36
The following were the enumeration results for the Control Treated Carriers for Decon7 Part 1, Part 2, and Booster (Lots: 16-15, 16-18, 470572802) tested on 10 FEB 2016 against *K. pneumoniae* ATCC 4352.

Test Microorganism	Test Substance	Control	CFU/Carrier	Mean CFU/Carrier	Mean Log_{10} Density
K. pneumoniae ATCC 4352	Lots: 16-15, 16-18, 470572802	Carriers	7.60E+05	8.23E+05	5.92
			8.70E+05		
			8.40E+05		

RESULTS (cont.)

Table 37

The following were the enumeration results for the Control Wash Water for Decon7 Part 1, Part 2, and Booster (Lots: 16-15, 16-18, 470572802) tested on 10 FEB 2016 against *K. pneumoniae* ATCC 4352.

Test Microorganism	Test Substance	Control	CFU/ml	Mean CFU/ml	Mean Log_{10} Density
K. pneumoniae ATCC 4352	Lots: 16-15, 16-18, 470572802	Wash Water	9.80E+04	1.06E+05	5.03
			8.80E+04		
			1.32E+05		

Table 38

The following were the test results for Decon7 Part 1, Part 2, and Booster (Lots: 16-13, 16-16, 470572802) when tested against *K. pneumoniae* ATCC 4352 at a contact time not to exceed 9.5 minutes in the presence of 5% ± 0.1% (v/v) fetal bovine serum (FBS) artificial soil. Agitation parameters were 360° vertical orbit of 4-8 inches at 45-60 RPM, specifically verified at 57 RPM. The pH of Decon7 Part 1, Part 2, and Booster combined without hard water was 10.19, the final prepared test substance had a pH of 9.82. Test was conducted on 02 FEB 2016.

Test Microorganism	Test Substance	Treatment Time	Contact Temperature	Contents of Test Tube	Number of Test Tubes Analyzed	Number of Positive Neutralizer Test Tubes	Number of Confirmed Positive Neutralizer Test Tubes
K. pneumoniae ATCC 4352	Lots: 16-13, 16-16, 470572802	≤9.5 minutes	24.3°C (Room temp at start of contact)	Carriers	9	0	0
				Wash Water	9	0	0

RESULTS (cont.)

Table 39

The following were the test results for Decon7 Part 1, Part 2, and Booster (Lots: 16-14, 16-17, 470572802) when tested against *K. pneumoniae* ATCC 4352 at a contact time not to exceed 9.5 minutes in the presence of 5% ± 0.1% (v/v) fetal bovine serum (FBS) artificial soil. Agitation parameters were 360° vertical orbit of 4-8 inches at 45-60 RPM, specifically verified at 57.6 RPM. The pH of Decon7 Part 1, Part 2, and Booster combined without hard water was 9.88, the final prepared test substance had a pH of 9.76. Test was conducted on 04 FEB 2016.

Test Microorganism	Test Substance	Treatment Time	Contact Temperature	Contents of Test Tube	Number of Test Tubes Analyzed	Number of Positive Neutralizer Test Tubes	Number of Confirmed Positive Neutralizer Test Tubes
K. pneumoniae ATCC 4352	Lots: 16-14, 16-17, 470572802	≤9.5 minutes	23.9°C (Room temp at start of contact)	Carriers	9	0	0
				Wash Water	9	0	0

Table 40

The following were the test results for Decon7 Part 1, Part 2, and Booster (Lots: 16-15, 16-18, 470572802) when tested against *K. pneumoniae* ATCC 4352 at a contact time not to exceed 10 minutes in the presence of 5% ± 0.1% (v/v) fetal bovine serum (FBS) artificial soil. Agitation parameters were 360° vertical orbit of 4-8 inches at 45-60 RPM, specifically verified at 52 RPM. The pH of Decon7 Part 1, Part 2, and Booster combined without hard water was 10.11, the final prepared test substance had a pH of 9.69. Test was conducted on 10 FEB 2016.

Test Microorganism	Test Substance	Treatment Time	Contact Temperature	Contents of Test Tube	Number of Test Tubes Analyzed	Number of Positive Neutralizer Test Tubes	Number of Confirmed Positive Neutralizer Test Tubes
K. pneumoniae ATCC 4352	Lots: 16-15, 16-18, 470572802	≤9.5 minutes	26.0°C (Room temp at start of contact)	Carriers	9	0	0
				Wash Water	9	0	0

RESULTS (cont.)

Table 41
The following were the neutralization results for Decon7 Part 1, Part 2, and Booster (Lots: 16-13, 16-16, 470572802) performed on 02 FEB 2016 against *K. pneumoniae* ATCC 4352. Neutralization results are in compliance with the aforementioned study acceptance criteria. The parallel neutralization broth control tube demonstrated positive growth indicative of the target microorganism.

Test Microorganism	Test Substance	Average Inoculum Concentration	Neutralization Verification Result
K. pneumoniae ATCC 4352	Lots: 16-13, 16-16, 470572802	67.5 CFU	Positive Growth, Valid

Table 42
The following were the neutralization results for Decon7 Part 1, Part 2, and Booster (Lots: 16-14, 16-17, 470572802) performed on 04 FEB 2016 against *K. pneumoniae* ATCC 4352. Neutralization results are in compliance with the aforementioned study acceptance criteria. The parallel neutralization broth control tube demonstrated positive growth indicative of the target microorganism.

Test Microorganism	Test Substance	Average Inoculum Concentration	Neutralization Verification Result
K. pneumoniae ATCC 4352	Lots: 16-14, 16-17, 470572802	62.5 CFU	Positive Growth, Valid

RESULTS (cont.)

Table 43

The following were the neutralization results for Decon7 Part 1, Part 2, and Booster (Lots: 16-15, 16-18, 470572802) performed on 10 FEB 2016 against *K. pneumoniae* ATCC 4352. Neutralization results are in compliance with the aforementioned study acceptance criteria. The parallel neutralization broth control tube demonstrated positive growth indicative of the target microorganism.

Test Microorganism	Test Substance	Average Inoculum Concentration	Neutralization Verification Result
K. pneumoniae ATCC 4352	Lots: 16-15, 16-18, 470572802	36.5 CFU	Positive Growth, Valid

Table 44

The following were the test microorganism culture incubation conditions and durations used for Decon7 Part 1, Part 2, and Booster (Lots: 16-13, 16-16, 470572802) tested on 02 FEB 2016 against *K. pneumoniae* ATCC 4352.

Test Microorganism	Transfer Date and Time	Incubation Temperature	Test Culture Transfer	Incubation Time
K. pneumoniae ATCC 4352	29 JAN 2016 / 1320	36 ± 1°C	1	20 hours 19 minutes
	30 JAN 2016 / 0939		2	23 hours 49 minutes
	31 JAN 2016 / 0928		3	30 hours 53 minutes
	01 FEB 2016 / 1621		4	18 hours 19 minutes

RESULTS (cont.)

Table 45
The following were the test microorganism culture incubation conditions and durations used for Decon7 Part 1, Part 2, and Booster (Lots: 16-14, 16-17, 470572802) tested on 04 FEB 2016 against *K. pneumoniae* ATCC 4352.

Test Microorganism	Transfer Date and Time	Incubation Temperature	Test Culture Transfer	Incubation Time
K. pneumoniae ATCC 4352	29 JAN 2016 / 1320	36 ± 1°C	1	20 hours 19 minutes
	30 JAN 2016 / 0939		2	23 hours 49 minutes
	31 JAN 2016 / 0928		3	30 hours 53 minutes
	01 FEB 2016 / 1623		4	24 hours 50 minutes
	02 FEB 2016 / 1713		5	24 hours 1 minute
	03 FEB 2016 / 1714		6	18 hours 35 minutes

Table 46
The following were the test microorganism culture incubation conditions and durations used for Decon7 Part 1, Part 2, and Booster (Lots: 16-15, 16-18, 470572802) tested on 10 FEB 2016 against *K. pneumoniae* ATCC 4352.

Test Microorganism	Transfer Date and Time	Incubation Temperature	Test Culture Transfer	Incubation Time
K. pneumoniae ATCC 4352	04 FEB 2016 / 1513	36 ± 1°C	1	22 hours 54 minutes
	05 FEB 2016 / 1407		2	25 hours 52 minutes
	06 FEB 2016 / 1559		3	22 hours 47 minutes
	07 FEB 2016 / 1446		4	25 hours 52 minutes
	08 FEB 2016 / 1638		5	22 hours 52 minutes
	09 FEB 2016 / 1530		6	21 hours 29 minutes

RESULTS (cont.)

Table 47
The following were the results for sterility, growth, and purity controls conducted during the study on 02 FEB 2016, 04 FEB 2016 and 10 FEB 2016 against *K. pneumoniae* ATCC 4352.

Study Controls	Result
Carrier Sterility Control Tube	No Growth Observed
Viability Control Tube	Growth-Target Microorganism
Neutralization Media Control Tube	No Growth Observed
Growth Media Control Plate	No Growth Observed
Culture Dilution Media Plate	No Growth Observed
Soil Sterility Control Plate	No Growth Observed
PBS Sterility Control Plate	No Growth Observed
AOAC Hard Water Sterility Control Plate	No Growth Observed
Microorganism Purity Plate	Pure-Target Microorganism

RESULTS (cont.)

Table 48

The following were the incubation times and temperature ranges for the test materials incubated for Decon7 Part 1, Part 2, and Booster (Lots: 16-13, 16-16, 470572802) tested on 02 FEB 2016 against *K. pneumoniae* ATCC 4352.

Test Microorganism	Test Materials	Incubation Temperature	Date / Time to Incubator	Incubation Time
K. pneumoniae ATCC 4352	NV Test and Control Tubes, Test Tubes, Carrier Sterility, Media Sterility, Viability Tubes, Enumeration Plates	36 ± 1°C	02 FEB 2016 / 1327	48 hours 37 minutes
	Confirmation Streak Plates		04 FEB 2016 / 1431	21 hours 54 minutes

Table 49

The following were the incubation times and temperature ranges for the test materials incubated for Decon7 Part 1, Part 2, and Booster (Lots: 16-14, 16-17, 470572802) tested on 04 FEB 2016 against *K. pneumoniae* ATCC 4352.

Test Microorganism	Test Materials	Incubation Temperature	Date / Time to Incubator	Incubation Time
K. pneumoniae ATCC 4352	NV Test and Control Tubes, Test Tubes, Carrier Sterility, Media Sterility, Viability Tubes, Enumeration Plates	36 ± 1°C	04 FEB 2016 / 1455	48 hours 26 minutes
	Confirmation Streak Plates		06 FEB 2016 / 1546	22 hours 53 minutes

RESULTS (cont.)

Table 50

The following were the incubation times and temperature ranges for the test materials incubated for Decon7 Part 1, Part 2, and Booster (Lots: 16-15, 16-18, 470572802) tested on 10 FEB 2016 against *K. pneumoniae* ATCC 4352.

Test Microorganism	Test Materials	Incubation Temperature	Date / Time to Incubator	Incubation Time
K. pneumoniae ATCC 4352	NV Test and Control Tubes, Test Tubes, Carrier Sterility, Media Sterility, Viability Tubes, Enumeration Plates	36 ± 1°C	10 FEB 2016 / 1604	48 hours 57 minutes
	Confirmation Streak Plates		12 FEB 2016 / 1728	23 hours 11 minutes

STUDY CONCLUSION

For study identification number GLP1362, test substance Decon7 Part 1 (Lots: 16-13, 16-14, and 16-15), Decon7 Part 2 (Lots: 16-16, 16-17, and 16-18), and Booster (Lot: 470572802) was tested against *Staphylococcus aureus* ATCC 6538 and *Klebsiella pneumoniae* ATCC 4352. A total of 9 contaminated carriers per lot were exposed to the test substance for a contact time of ≤9.5 minutes or ≤10 minutes and then chemically neutralized. In addition to neutralizing carriers, 9 aliquots of wash water per lot were neutralized after exposure to the test system.

Test substance Decon7 Lot: 16-13, 16-16, 470572802 disinfected 9 out of 9 carriers and 9 out of 9 wash water aliquots containing *S. aureus* ATCC 6538 within 9.5 minutes.
Test substance Decon7 Lot: 16-14, 16-17, 470572802 disinfected 9 out of 9 carriers and 9 out of 9 wash water aliquots containing *S. aureus* ATCC 6538 within 9.5 minutes.
Test substance Decon7 Lot: 16-15, 16-18, 470572802 disinfected 9 out of 9 carriers and 9 out of 9 wash water aliquots containing *S. aureus* ATCC 6538 within 10 minutes.

Test substance Decon7 Lot: 16-13, 16-16, 470572802 disinfected 9 out of 9 carriers and 9 out of 9 wash water aliquots containing *K. pneumoniae* ATCC 4352 within 9.5 minutes.
Test substance Decon7 Lot: 16-14, 16-17, 470572802 disinfected 9 out of 9 carriers and 9 out of 9 wash water aliquots containing *K. pneumoniae* ATCC 4352 within 9.5 minutes.
Test substance Decon7 Lot: 16-15, 16-18, 470572802 disinfected 9 out of 9 carriers and 9 out of 9 wash water aliquots containing *K. pneumoniae* ATCC 4352 within 9.5 minutes.

The test substance Decon7 Part 1 (Lots: 16-13, 16-14, and 16-15), Decon7 Part 2 (Lots: 16-16, 16-17, and 16-18), and Booster (Lot: 470572802) met the U.S. EPA Product Performance Guidelines for Disinfectants and Sanitizers for Use on Fabrics and Textiles outlined in OCSPP 810.2400.

The study was carried out in compliance with the approved protocol (P1428) except where noted on page 10-11 of this report.

REFERENCES

- "ASTM, International." *ASTM Official Method E2274-09*. Standard Test Method for Evaluation of Laundry Sanitizers and Disinfectants. 2009.
- U.S. EPA Product Performance Test Guidelines OCSPP 810.2400: Disinfectants and Sanitizers for Use on Fabrics and Textiles —Efficacy Data Recommendations

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 Dimethylbenzyl 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 (cont.)

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 BCQC8P – 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						

PROTOCOL

Title
Protocol for Testing Disinfectants Under Simulated Industrial Laundry Conditions

Test Microorganisms
Staphylococcus aureus ATCC 6538
Klebsiella pneumoniae ATCC 4352

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

Data Requirement
US EPA 40 CFR Part 158
U.S. EPA OCSPP 810.2400

Study Sponsor
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Protocol Number
P1428

Study Director
Elizabeth Richard, B.S.

PROTOCOL (cont.)

I. Introduction

This document details the materials and procedure for evaluating the antimicrobial efficacy of a disinfecting laundry detergent formula using the ASTM E2274 Standard Test Method for disinfection and guidance found in U.S. EPA OCSPP 810.2400 for Evaluation of Laundry Disinfectants 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 (microorganisms) under the test parameters specified in this protocol.

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

The test microorganisms listed on page 1 of this protocol are recommended for use in ASTM E2274 as well as designated for testing per EPA Product Performance Test Guidelines, OCSPP 810.2400.

IV. Terms and Conditions

Studies by Microchem Laboratory are conducted in accordance with general terms and conditions posted on our website.

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., (40 CFR, Part 160, and Subpart 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.

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) and certificates of analysis will be provided by the Study Sponsor to the Test Facility prior to completion of this study.

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

Test substance characterization as to content, stability, etc., (40 CFR, Part 160, and Subpart 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, and such data shall be maintained by the Study Sponsor.

Test substances are handled as follows:

- The test substance is stored at ambient (room) temperature under fluorescent lighting or in a cabinet.
- 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 (cont.)

VI. Study Parameters, Incorporated by Reference

Number of Tests Comprising This Study — 6 (1 test per test substance lot per test microorganism)
Carrier Type — Cotton fabric (1 in. x 1.5 in.)
Numbers Control Carrier Replicates — 3 carriers (i.e. 1 spindle) per test microorganism per lot
Carrier Count Control Replicates — 3 carriers per test microorganism per test substance lot
Test Carrier Replicates — 9 carriers (3 spindles, 3 carriers per spindle) per test substance lot per test microorganism
Test Substance Form — Dilution required (1:1:42), e.g. 1 part Decon7 Part 1 + 1 part Decon7 Part 2 + 20 parts diluent/Booster
Fabric to Wash Water Ratio — 1:5 (e.g. 15.0 ± 0.1 g fabric to 75.0 ± 0.1 ml dilute test substance)
Test Substance Diluent — 200 ppm ± 10 ppm AOAC Synthetic Hard Water
Control Substance — 200 ppm ± 10 ppm AOAC Synthetic Hard Water supplemented with 0.5% (v/v) Triton X-100
Contact Time — ≤9.5 minutes
Test Temperature — Ambient temperature, to be recorded in final report
Organic Soil Load — 5% (v/v) Fetal Bovine Serum
Neutralization Broth — Dey Engley Neutralization Broth supplemented with 0.1% Catalase

Proposed Experimental Start Date: 29JAN2016
Proposed Experimental Termination Date: 05FEB2016

VII. Test System (Microorganism)

Staphylococcus aureus ATCC 6538
Klebsiella pneumoniae ATCC 4352

VIII. Materials

Reagents, Media, and Supplies:

- Pure culture of test systems (microorganisms)
- Sufficient quantity of 100% cotton fabric
- Sufficient quantity of sterile glass exposure chambers (Mason jars or equivalent)
- Sufficient quantity of Petri dishes containing sterile Nutrient Agar A
- Sufficient quantity of sterile Nutrient Agar B
- Sufficient quantity of sodium carbonate
- Sufficient quantity of sterile glass beads
- Sufficient quantity of sterile Petri dishes
- Sufficient volume of sterile Tryptic Soy Agar
- Sufficient quantity of sterile neutralization broth
- Sufficient volume of sterile 200 ± 10 ppm AOAC Synthetic Hard Water
- Sufficient quantity of sterile Phosphate Buffered Saline (PBS)
- Sufficient quantity of sterile stainless steel spindles as described in ASTM E2274
- Sufficient volume of Fetal Bovine Serum (FBS)
- Sufficient volume of Triton X-100
- Sufficient quantity of calibrated micropipettes and appropriately sized sterile micropipette tips
- Appropriate volume of 95% ethanol
- Sufficient quantity of forceps
- Inoculating loop (microbiological loop)
- Bunsen burner, microbiological incinerator, or micro-torch as appropriate

PROTOCOL (cont.)

- Automatic pipettor (PipetAid or similar) and various sizes of sterile serological pipettes
- Sufficient quantity of sterile 50 ml centrifuge tubes containing sterile neutralizing broth
- Sufficient number of test tube racks
- Certified satellite clock
- Calibrated digital timer
- Calibrated hygrometer
- Calibrated pH meter
- Agitator capable of 360° vertical orbit of 4 to 8 inches at 45 – 60 rpm
- Incubators capable of sustaining temperatures of 36° ± 1° C
- Balance
- Clock
- Vortex Mixer

IX. Procedure

Preparation of AOAC synthetic hard water solution

- From each 1000 ml of sterile RO water (as measured by 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 to determine the volume of solution "1". That is the volume, in ml, of AOAC hard water solution "1" that is needed to make 1000ml of hard water. For example, if the requested concentration is 400 ppm, 4 ml of solution "1" are required.
- 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 equal parts 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 Neutralization / Elution Media

- Before the test begins a sufficient volume of neutralization broth is prepared and steam sterilized prior to use.

Preparation of Test Fabric

- Test fabric is scoured by boiling approximately 300 g of material for 1 hour in 3 liters of reverse osmosis water, containing 1.5 g of sodium carbonate and 1.5 ml of Triton X-100.
- After scouring, test fabric is rinsed, first in boiling water then in cold water, until all visual traces of the wetting agent is removed.
- Test fabric is allowed to air dry for at least 24 hours at ambient room temperature before manipulation.

Preparation of Spindles

- Stainless steel spindles are fabricated from a single continuous piece of stainless steel wire, 1/16" diameter and bent to contain 3 horizontal extensions, approximately 2 in. long connected by 2 vertical sections approximately 2 in. long. Spindles are shaped so that vertical sections form an approximate 150° angle.

PROTOCOL (cont.)

- Dried test fabric is cut into 2 in. wide strips weighing 15.0 ± 0.1 g per strip.
- A strip of test fabric is then pierced onto one end of a spindle to secure fabric and to allow for the winding of fabric around spindle. The fabric is then wrapped around the three horizontal extensions of the spindle with sufficient tension to obtain 12 wraps.
- A staple is used to secure the fabric in place. Each fabric wrapped spindle is steam sterilized in its appropriate exposure chamber and allowed to fully dry before testing. Fabric drying may be facilitated by incubation at elevated temperatures.
- Fabric and exposure chambers may be steam sterilized separately to ensure proper drying.

Preparation of Fabric Carriers

- Fabric carriers of approximately 1 in. x 1.5 in. are cut from the remaining processed fabric and one end of each strip is marked with non-toxic marker or a pin is fixed to the short side of each carrier.
- Carriers are steam sterilized and dried prior to use.

Preparation of Test Culture

- A daily culture is initiated from the monthly working stock culture or from the frozen microbial library for each microorganism to be tested. Each daily transfer is subcultured to a petri dish containing solidified Nutrient Agar A and incubated for 24 ± 6 hours at 36° ± 1° C.
- Daily cultures of each test microorganism are transferred at least three times consecutively prior to use as the test culture; one missed transfer does not require starting the series over.
- To initiate the test culture, the microorganism is removed from the daily culture's agar surface by rinsing the entire plate with 5 ml of sterile phosphate buffered saline and diluting the 5 ml of culture removed from the plate with 99 ml of sterile phosphate buffered saline. A sufficient number of plates containing solidified Nutrient Agar B are inoculated with 0.2 ml from the prepared inoculum which is spread evenly over the entire surface of agar. The prepared test culture plates are incubated for 18 to 24 hours at 36° ± 1° C.
- Surface growth from the test culture plate is suspended using 3 ml of sterile phosphate buffered saline. Cell removal may be assisted by glass beads or a cell scraper. Volumes from each test culture dish are pooled in a sterile vessel and vortex mixed.
- Cultures are diluted to yield approximately 1.0×10^8 CFU/ml for *Staphylococcus aureus* and approximately 1.0×10^7 for *Klebsiella pneumoniae*. Diluted cultures are used as test inoculum.
- Thawed, sterile Fetal Bovine Serum is added to each test culture after dilution such that the final concentration is 5.0 ± 0.1% (v/v).

Preparation of Test Substance

- Prior to wash water preparation, equal parts of Decon 7 Part 1, Decon 7 Part 2, and 2 ± 0.1% Booster are combined and a pH reading is taken.
- Test substance (i.e. wash water) is prepared by dilution (1:1:42), specifically 1 part of Decon 7 Part 1 to 1 part Decon 7 Part 2 to 40 parts of sterile 200 ± 10 ppm AOAC Synthetic Hard Water/Booster.
- Test substance is prepared by mixing 23.80ml of Part 1, 23.80ml of Part 2, 0.97ml of Booster, and 951.43ml of diluent to make 1 liter of test substance. Other proportional volumes are used as necessary for the conduct of this test. Enough test substance is prepared to facilitate the volume required for testing.
- The test substance is allowed to rest at ambient temperature for ≥ 10 minutes prior to use in this study. A pH reading is taken of the final test substance.
- The prepared test substance (i.e. wash water) is used within 3 hours of conclusion of the rest period.

PROTOCOL (cont.)

Fabric Carrier Inoculation with Test Culture

- Using a calibrated micropipette, 0.010 – 0.030 ml of test culture are used to inoculate the entire surface of each fabric carrier, while avoiding the marked end (i.e. marker or safety pin).
- All inoculated fabric carriers are incubated at 36 ± 1°C until visibly dry, but not longer than 30 minutes.
- Fabric carriers are used within 1 hour of drying.

Test Culture Enumeration

- Each culture used for inoculation of carriers is serially diluted 1:10 in sterile phosphate buffered saline and plated to determine the initial concentration in CFU/ml (CFU = Colony Forming Unit).

Carrier Count Control

- Three inoculated fabric carriers, per microorganism per lot of test substance, are individually harvested into neutralization broth, vortex mixed for 120 ± 5 seconds to elute test microorganisms, serially diluted 1:10 in sterile phosphate buffered saline then plated to determine the initial concentration in CFU/carrier (CFU = Colony Forming Units).

Preparation of Exposure Chambers with Control Substance and Spindles

- Three dried inoculated fabric carriers, per microorganism per lot, are aseptically placed in an upright position between the sixth and seventh folds of a single fabric wrapped spindle, without allowing carriers to overlap.
- 75.0 ml of 200 ± 10 ppm AOAC Synthetic Hard Water supplemented with 0.5% (v/v) Triton X-100 is added to each exposure chamber.
- Each fabric wrapped spindle is aseptically placed in the appropriate sterile exposure chamber initiating the contact time. The contact initiation time of control substance with carriers is recorded.
- The exposure chamber is firmly closed and placed on the laundry agitator.
- Chambers are tumbled via 360° vertical orbit, 4-8 inch diameter at 45-60 rpm. The duration of agitation and the contact time is recorded.

Preparation of Exposure Chambers with Test Substance and Spindles

- Test substance is prepared for testing as described in the *Preparation of Test Substance* section of this protocol.
- Three dried inoculated fabric carriers, per microorganism, are aseptically placed in an upright position between the sixth and seventh folds of a single fabric wrapped spindle, without allowing carriers to overlap.
- A total of three spindles are required per test substance lot per test microorganism, this requires three separate exposure chambers.
- 75.0 ml of prepared test substance are added to each exposure chamber.
- Each fabric wrapped spindle is aseptically placed in the appropriate sterile exposure chamber initiating the contact time. The contact initiation time of test substance with carriers is recorded.
- The exposure chamber is firmly closed and placed on the laundry agitator.
- Chambers are tumbled via 360° vertical orbit, 4-8 inch diameter at 45-60 rpm. The duration of agitation and the contact time is recorded.

PROTOCOL (cont.)

Harvesting Carriers from Control and Test Substances

- Agitation is stopped prior to the contact time to allow for carrier and wash water harvesting by the designated contact time.
- Carriers are harvested at intervals before 9.5 minutes (for example: the first carrier at 9 minutes and 10 seconds, the second carrier at 9 minutes and 20 seconds, and the third carrier at 9 minutes and 30 seconds). All harvest times are recorded.
- The spindles are removed from the exposure chamber, carriers are harvested using flame sterilized forceps. Each fabric carrier is aseptically removed from the folds of the fabric spindle and placed into a tube containing 20 ml of neutralizing broth. Tubes containing test fabric carriers are vortex mixed for approximately 10 seconds. Neutralizer tubes containing control carriers are vortex mixed for 120 ± 5 seconds.
- This process is repeated until all control and test carriers are neutralized.
- In addition to harvesting the fabric carriers, three 0.5 ml volumes of wash water per exposure chamber for both control and test substances are transferred directly to 19.5 ml neutralization broth and thoroughly vortex mixed.
- The neutralizer from control carriers and control wash water are enumerated in duplicate, using standard dilution and plating techniques to determine CFU/carrier and CFU/ml, respectively.

X. Controls

Carrier Sterility Control(s)

- A single uninoculated carrier is placed in a conical tube containing 20 ml of neutralization media and gently vortex mixed. This tube is incubated alongside test tubes.

Carrier Viability Control

- One inoculated carrier per microorganism is placed in individual subculture/neutralization broth tubes and incubated alongside test.

Media Sterility Control(s)

- Aliquots of each media type used in the study (e.g. phosphate buffered saline, neutralization broth, enumeration media) are plated to determine media sterility. One plate containing only the growth medium is incubated to determine media sterility.

"Soil" Sterility Control

- An aliquot of soil is plated to sterile growth medium and incubated alongside test to verify sterility at time of test.

Media Viability and Culture Purity Control

- A loop full of each test microorganism culture is struck to the appropriate growth agar to achieve isolated colonies to confirm culture purity and media viability.

PROTOCOL (cont.)

Neutralization Confirmation

- Three individual sterile carriers, per test microorganism per test substance lot, are assembled in a sterile fabric wrapped spindle and treated in the same manner as test carriers as described in the section titled *Preparation of Exposure Chambers with Test Substance and Spindles* in this protocol.
- Carriers and three 0.5 ml aliquots of wash water, per microorganism per test substance lot, are harvested as described in *Harvesting Carriers from Control and Test Substances* as it relates to the test substance carriers.
- All neutralizer tubes are inoculated with 0.100 ml of dilute test microorganism (≤100 CFU) obtained by serial dilution in phosphate buffered saline.
- Tubes are vortex mixed and the inoculum plated in duplicate to determine total CFU.
- Neutralization tubes and enumeration plates are incubated alongside test.
- Neutralization is confirmed by presence of turbidity or colorimetric change (purple to yellow) after the incubation time.

XI. Incubation of Plates, Tubes and Controls

- All plates and tubes are incubated for 48 to 54 hours at 36° ± 1° C.

XII. Confirmation of Positive Tubes

- Tubes demonstrating growth are subcultured to growth media alongside control or viability tubes to confirm presence of test microorganism morphology, incubated for 18-24 hours, and date recorded.

XIII. Calculations

- Results are reported as growth (+) or no growth (-) for each plate and tube for disinfectant efficacy.
- CFU/Carrier = Average CFU/plate x dilution factor (relative to carrier)
- CFU/ml= Average CFU/plate x dilution factor (relative to volume)

XIV. Success Criteria

- The experimental success (controls) criteria follow:
  1. All media sterility controls must be negative for growth..
  2. Carrier sterility control must be negative for growth.
  3. Carrier viability control is positive for growth.
  4. The media viability control must be positive for growth.
  5. All test microorganisms must demonstrate culture purity.
  6. Neutralization validation tubes, test and controls, demonstrate turbidity (growth) of test microorganism and the inoculum enumeration yields ≤100 CFU.
  7. An average of at least 1.0×10^5 CFU/carrier must be recovered from the inoculated washed control fabric carriers and an average of at least 1.0×10^5 CFU/ml must be recovered from the inoculated wash water treated with the control solution.

PROTOCOL (cont.)

XV. 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.

XVI. Data and Sample Retention

* The study report, and corresponding data will be held in the archives of Microchem Laboratory for at least 2 years after the date of the final report. After 2 years, documentation may be returned to the Study Sponsor for archiving.
* The test substance may be returned to the Study Sponsor at 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 >30 days after study completion. Archiving of test substances is the responsibility of the Sponsor.

XVII. Quality Control

* The study will be conducted in accordance with the Performing Laboratories 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.

XVIII. References

1. "ASTM International" *ASTM Official Method E2274-09*. Standard Test Method for Evaluation of laundry Sanitizers and Disinfectants.
2. US EPA Product Performance Test Guidelines OCSPP 810.2400: Disinfectants and Sanitizers for Use on Fabrics and Textiles-Efficacy Data Recommendations

XIX. 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 Part 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, Sponsor, Decon7 Systems, LLC — Date: 1/29/2016

Elizabeth Richard, B.S., Study Director, Microchem Laboratory — Study Initiation Date: 03FEB2016

PROTOCOL AMENDMENTS

Protocol Amendment
Protocol for Testing Disinfectants Under Simulated Industrial Laundry Conditions
P1428
Page 1 of 1

Amendment 1:

The signed protocol (P1363) is hereby amended to include the following change in section VI:

"Contact Time – ≤9.5 Minutes"

is amended to

"Contact Time – ≤10 Minutes"

"Carriers are harvested at intervals before 9.5 minutes (for example: the first carrier at 9 minutes and 10 seconds, the second carrier at 9 minutes and 20 seconds, and the third carrier at 9 minutes and 30 seconds). All harvest times are recorded."

is amended to

"Carriers are harvested at intervals before 10 minutes (for example: the first carrier at 9 minutes and 40 seconds, the second carrier at 9 minutes and 50 seconds, and the third carrier at 10 minutes). All harvest times are recorded."

All other testing parameters not mentioned in this amendment are to remain in place for testing.

"I, the Study Sponsor, have read, understand, and agree to the aforementioned amendment(s) to protocol P1428."

Joseph Drake — 2/15/2016

Role: Study Sponsor
Name: Joe Drake
Company: Decon7 Systems, LLC
Address: 7575 E. Redfield Rd., Suite 235, Scottsdale, AZ 85260

Date (dd/mmm/yyyy)

Elizabeth Richard — 15 FEB 2016

Role: Study Director
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PROTOCOL AMENDMENTS (cont.)

Protocol Amendment
Protocol for Testing Disinfectants Under Simulated Industrial Laundry Conditions
P1428
Page 1 of 1

Amendment 2:

The signed protocol (P1428) is hereby amended to include the following change in the entire document:

The lot number for Decon7 Booster is amended from 4705722802 to 470572802.

All other testing parameters not mentioned in this amendment are to remain in place for testing.

"I, the Study Sponsor, have read, understand, and agree to the aforementioned amendment(s) to protocol P1428."

Role: Study Sponsor
Name: Joe Drake
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3/3/2016
Date (dd/mmm/yyyy)

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APPENDIX – A

Table A-1

The following were the neutralization results for Decon7 Part 1, Part 2, and Booster (Lots: 16-13, 16-16, 470572802) performed on 02 FEB 2016 against *S. aureus* ATCC 6538. Neutralization results were not in compliance with the aforementioned study acceptance criteria. The neutralization verification for this lot was repeated on 08 FEB 2016 as reported on Page 22 in Table 16.

Test Microorganism	Test Substance	Average Inoculum Concentration	Neutralization Verification Result
S. aureus ATCC 6538	Lots: 16-13, 16-16, 470572802	289.5 CFU	Positive Growth, Invalid

APPENDIX – B

Table B-1
The following were the enumeration results for the inoculum for Decon7 Part 1, Part 2, and Booster (Lots: 16-15, 16-18, 470572802) against *S. aureus* ATCC 6538 tested on 10 FEB 2016.

Test Microorganism	Test Substance	Control and Dilution	Mean CFU/ml	Mean Log_{10} Density
S. aureus ATCC 6538	Lots: 16-15, 16-18, 470572802	Inoculum 1:50	2.90E+08	8.46

Table B-2
The following were the enumeration results for the Carrier Count Control for Decon7 Part 1, Part 2, and Booster (Lots: 16-15, 16-18, 470572802) against *S. aureus* ATCC 6538 tested on 10 FEB 2016.

Test Microorganism	Test Substance	Control	CFU/Carrier	Mean CFU/Carrier	Mean Log_{10} Density
S. aureus ATCC 6538	Lots: 16-15, 16-18, 470572802	Carrier Count Control	5.50E+06	5.47E+06	6.74
			5.40E+06		
			5.50E+06		

Table B-3
The following were the enumeration results for the Control Treated Carriers for Decon7 Part 1, Part 2, and Booster (Lots: 16-15, 16-18, 470572802) against *S. aureus* ATCC 6538 tested on 10 FEB 2016.

Test Microorganism	Test Substance	Control	CFU/Carrier	Mean CFU/Carrier	Mean Log_{10} Density
S. aureus ATCC 6538	Lots: 16-15, 16-18, 470572802	Carriers	4.02E+06	4.10E+06	6.61
			3.85E+06		
			4.43E+06		

APPENDIX – B (cont.)

Table B-4
The following were the enumeration results for the Control Wash Water for Decon7 Part 1, Part 2, and Booster (Lots: 16-15, 16-18, 470572802) against *S. aureus* ATCC 6538 tested on 10 FEB 2016.

Test Microorganism	Test Substance	Control	CFU/ml	Mean CFU/ml	Mean Log_{10} Density
S. aureus ATCC 6538	Lots: 16-15, 16-18, 470572802	Wash Water	1.18E+04	1.23E+04	4.09
			1.04E+04		
			1.46E+04		

Table B-5
The following were the test results for Decon7 Part 1, Part 2, and Booster (Lots: 16-15, 16-18, 470572802) when tested against *S. aureus* ATCC 6538 at a contact time not to exceed 9.5 minutes in the presence of 5% ± 0.1% (v/v) fetal bovine serum (FBS) artificial soil. Agitation parameters were 360° vertical orbit of 4-8 inches at 45-60 RPM, specifically verified at 54 RPM. The pH of Decon7 Part 1, Part 2, and Booster combined without hard water was 10.11, the final prepared test substance had a pH of 9.69. Test was conducted on 10 FEB 2016.

Test Microorganism	Test Substance	Treatment Time	Contact Temperature	Contents of Test Tube	Number of Test Tubes Analyzed	Number of Positive Neutralizer Test Tubes	Number of Confirmed Positive Neutralizer Test Tubes
S. aureus ATCC 6538	Lots: 16-15, 16-18, 470572802	≤9.5 minutes	26.8°C (Room temp at start of contact)	Carriers	9	1	1
				Wash Water	9	0	0

APPENDIX – B (cont.)

Table B-6

The following were the neutralization results for Decon7 Part 1, Part 2, and Booster (Lots: 16-13, 16-16, 470572802) against *S. aureus* ATCC 6538 performed on 02 FEB 2016. Neutralization results were in compliance with the aforementioned study acceptance criteria.

Test Microorganism	Test Substance	Average Inoculum Concentration	Neutralization Verification Result
S. aureus ATCC 6538	Lots: 16-15, 16-18, 470572802	21 CFU	Positive Growth, Valid

Table B-7

The following were the results for sterility, growth, and purity controls conducted during the study on 02 FEB 2016, 04 FEB 2016 and 10 FEB 2016 against *S. aureus* ATCC 6538.

Study Controls	Result
Carrier Sterility Control Tube	No Growth Observed
Viability Control Tube	Growth-Target Microorganism
Neutralization Media Control Tube	No Growth Observed
Growth Media Control Plate	No Growth Observed
Culture Dilution Media Plate	No Growth Observed
Soil Sterility Control Plate	No Growth Observed
PBS Sterility Control Plate	No Growth Observed
AOAC Hard Water Sterility Control Plate	No Growth Observed
Microorganism Purity Plate	Pure-Target Microorganism

APPENDIX – B (cont.)

Table B-8

The following were the incubation times and temperature ranges for the test materials incubated for Decon7 Part 1, Part 2, and Booster (Lots: 16-13, 16-16, 470572802) against *S. aureus* ATCC 6538 tested on 10 FEB 2016.

Test Microorganism	Test Materials	Incubation Temperature	Date / Time to Incubator	Incubation Time
S. aureus ATCC 6538	NV Test and Control Tubes, Test Tubes, Carrier Sterility, Media Sterility, Viability Tubes, Enumeration Plates	36 ± 1°C	10 FEB 2016 / 1656	48 hours 34 minutes
	Confirmation Streak Plates		12 FEB 2016 / 1752	22 hours 44 minutes