FINAL STUDY REPORT

STUDY TITLE

Germicidal and Detergent Sanitizing Action of Disinfectants

Test Organism(s):

Escherichia coli (ATCC 11229)
Staphylococcus aureus (ATCC 6538)

PRODUCT IDENTITY

D7 Part 1 Lot 16-353, Part 2 Lot 16-450, Part 3 Lot 18840
D7 Part 1 Lot 16-354, Part 2 Lot 16-451, Part 3 Lot 18840
D7 Part 1 Lot 16-355, Part 2 Lot 16-452, Part 3 Lot 18840

TEST GUIDELINE

OCSPP 810.2300

PROTOCOL NUMBER

DSS01072816.GDST

AUTHOR

Jamie Herzan, B.S.
Study Director

STUDY COMPLETION DATE

December 15, 2016

PERFORMING LABORATORY

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

SPONSOR

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

PROJECT NUMBER

A21809

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality, on any basis whatsoever, is made for any information contained in this document. I acknowledge that information not designated as within the scope of FIFRA sec. 10(d)(1)(A), (B), or (C) and which pertains to a registered or previously registered pesticide is not entitled to confidential treatment and may be released to the public, subject to the provisions regarding disclosure to multinational entities under FIFRA 10(g).

Company: Decon7 Systems, LLC

Company Agent: Joseph Drake

President
Title

Joseph Drake
Signature

Date: 12/15/2016

GOOD LABORATORY PRACTICE STATEMENT

The study referenced in this report was conducted in compliance with U.S. Environmental Protection Agency Good Laboratory Practice (GLP) regulations set forth in 40 CFR Part 160 with the following exceptions:

The following studies were not performed following GLP regulations: characterization and stability of the compounds.

Submitter:_____ Date:_____

Sponsor: Joseph Drake Date: 12/15/2016

Study Director: Jamie Herzan Date: 12-15-16
Jamie Herzan, B.S.

QUALITY ASSURANCE UNIT SUMMARY

Study: Germicidal and Detergent Sanitizing Action of Disinfectants

The objective of the Quality Assurance Unit is to monitor the conduct and reporting of non-clinical laboratory studies. This study has been performed in accordance to standard operating procedures and the study protocol. In accordance with Good Laboratory Practice regulation 40 CFR Part 160, the Quality Assurance Unit maintains a copy of the study protocol and standard operating procedures and has inspected this study on the date(s) listed below. Studies are inspected at time intervals to assure the integrity of the study. The findings of these inspections have been reported to Management and the Study Director.

Phase Inspected	Date of Phase Inspection	Date Reported to Study Director	Date Reported to Management
Critical Phase Audit: Exposure Conditions	October 10, 2016	October 10, 2016	October 12, 2016
Critical Phase Audit: Exposure Conditions	November 2, 2016	November 2, 2016	November 3, 2016
Critical Phase Audit: Exposure Conditions	December 6, 2016	December 6, 2016	December 8, 2016
Final Report	December 13, 2016	December 13, 2016	December 15, 2016

Quality Assurance Specialist: _____ Date: 12-15-16

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STUDY PERSONNEL

STUDY DIRECTOR: Jamie Herzan, B.S.

Professional personnel involved:

Becky Lien, B.A.	- Manager, Study Director Operations
Peter Toll, B.S.	- Manager, Microbiology Laboratory Operations
Kristen Niehaus, B.A.	- Supervisor, Microbiology Laboratory Operations
Adam W. Pitt, B.S.	- Lead Research Scientist
Maggie Brusky, B.S.	- Microbiologist
Melissa Bruner, M.S.	- Microbiologist
Andrea Epperly, B.S.	- Microbiologist
Kyle Kuras, B.S.	- Microbiologist
Thomas Casey, B.S.	- Microbiologist
T'Yanna Singleton, B.S.	- Associate Microbiologist
Matthew Kranz, B.S.	- Associate Microbiologist

STUDY REPORT

GENERAL STUDY INFORMATION

Study Title: Germicidal and Detergent Sanitizing Action of Disinfectants

Project Number: A21809

Protocol Number: DSS01072816.GDST

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

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

TEST SUBSTANCE IDENTITY

Test Substance Name: D7 Part 1 Lot 16-353, Part 2 Lot 16-450, Part 3 Lot 18840
D7 Part 1 Lot 16-354, Part 2 Lot 16-451, Part 3 Lot 18840
D7 Part 1 Lot 16-355, Part 2 Lot 16-452, Part 3 Lot 18840

Test Substance Characterization
Test substance characterization as to identity, strength, purity, solubility and composition, as applicable, was documented prior to its use in the study, however, not in accordance to 40 CFR, Part 160, Subpart F [160.105]. The Test Substance Certificate of Analysis Reports may be found in Attachments III-IV.

STUDY DATES

Date Sample Received: September 2, 2016 (Test Dates: 10/10/16 and 11/2/16) and December 6, 2016 (Test Date: 12/6/16)
Study Initiation Date: September 22, 2016
Experimental Start Date: October 10, 2016 (Start time: 9:52 am)
Experimental End Date: December 7, 2016 (End time: 3:30 pm)
Study Completion Date: December 15, 2016

OBJECTIVE

The objective of this assay was to determine the efficacy of a product to sanitize pre-cleaned, nonporous food contact surfaces using the AOAC Germicidal and Detergent Sanitizing Action of Disinfectants method. This method is in compliance with the requirements of the U.S. Environmental Protection Agency (EPA).

SUMMARY OF RESULTS

Test Substance:	D7 (Part 1 Lot 16-353, Part 2 Lot 16-450, Part 3 Lot 18840) D7 (Part 1 Lot 16-354, Part 2 Lot 16-451, Part 3 Lot 18840) D7 (Part 1 Lot 16-355, Part 2 Lot 16-452, Part 3 Lot 18840)
Dilution:	Equal parts Part 1 and Part 2 and 2% Part 3 defined as 49 oz Part 1 + 49 oz Part 2 + 2 oz Part 3
Test Organisms:	*Escherichia coli* (ATCC 11229) *Staphylococcus aureus* (ATCC 6538)
Exposure Time:	30 seconds
Exposure Temperature:	25±1°C (25.0°C)
Organic Soil Load:	No organic soil load required
Efficacy Result:	D7 demonstrated efficacy of three batches against *Escherichia coli*, and therefore, meets the performance requirements set forth by the U.S. Environmental Protection Agency following a 30 second exposure time at 25±1°C (25.0°C). D7 demonstrated efficacy of three batches against *Staphylococcus aureus*, and therefore, meets the performance requirements set forth by the U.S. Environmental Protection Agency following a 30 second exposure time at 25±1°C (25.0°C).

TEST HISTORY

Testing performed on October 10, 2016, resulted in a neutralization confirmation control failure for test substance, D7 Part 1 Lot 16-353, Part 2 Lot 16-450, Part 3 Lot 18840 against *Staphylococcus aureus*. Due to the neutralization confirmation control failure testing of D7 Part 1 Lot 16-353, Part 2 Lot 16-450, Part 3 Lot 18840 against *Staphylococcus aureus* was repeated on November 2, 2016 using a different neutralizer. Testing performed on November 2, 2016, resulted in another neutralization confirmation control failure for D7 Part 1 Lot 16-353, Part 2 Lot 16-450, Part 3 Lot 18840 against *Staphylococcus aureus*. Testing of D7 Part 1 Lot 16-353, Part 2 Lot 16-450, Part 3 Lot 18840 against *Staphylococcus aureus* was performed a third time on December 6, 2016 using a different neutralizer which resulted in valid results and are presented in the body of the report. Results for testing of D7 Part 1 Lot 16-353, Part 2 Lot 16-450, Part 3 Lot 18840 against *Staphylococcus aureus* performed on October 10, 2016 and November 2, 2016 are invalid and presented in Attachments I and II, respectively.

STUDY MATERIALS

Test System/Growth Media

Test Organism	Designation #	Growth Medium	Incubation Parameters
Escherichia coli	11229	Nutrient Agar A & B	35-37°C, aerobic
Staphylococcus aureus	6538	Nutrient Agar A & B	35-37°C, aerobic

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

Recovery Media:
Neutralizers:
D/E Neutralizing Broth + 0.01% Catalase (Test Date 10/10/16) and
D/E Neutralizing Broth + 0.28% Lecithin + 2.0% Tween 80 + 0.1% Sodium Thiosulfate + 0.1% Catalase (Test Date 12/6/16)

Subculture Agar Medium: Tryptic Soy Agar + 5% Sheep's Blood

TEST METHOD

Testing was performed the same on each test date, unless otherwise noted.

Preparation of Test Substance
For testing performed on 10/10/16, an equivalent dilution of equal parts Part 1 and Part 2 and 2% Part 3 defined as 49 oz Part 1 + 49 oz Part 2 + 2 oz Part 3 was prepared using 147.0 mL of Part 1, 147.0 mL of Part 2 and 6.0 mL of Part 3. The solution was mixed and allowed to stand for 5 minutes prior to use in testing. For testing performed on 12/6/16, an equivalent dilution of equal parts Part 1 and Part 2 and 2% Part 3 defined as 49 oz Part 1 + 49 oz Part 2 + 2 oz Part 3 was prepared using 98.0 mL of Part 1, 98.0 mL of Part 2 and 4.0 mL of Part 3. The solution was mixed and allowed to stand for 5 minutes prior to use in testing. The prepared test substance was homogenous as determined by visual observation and was used within three hours of preparation. A 99.0 mL aliquot of test substance was transferred to a sterile 250 – 300 mL Erlenmeyer flask per test organism, per lot. Each flask was placed into a water bath at 25.0°C and equilibrated for ≥10 minutes.

Preparation of Test Organisms

For *Staphylococcus aureus* (ATCC 6538) and *Escherichia coli* (ATCC 11229), a loopful of a thawed cryovial of stock organism broth culture was streaked to a Nutrient Agar A slant medium and was incubated at 35-37°C for 24±2 hours. For the final test culture, 5.0 mL of Phosphate Buffer Dilution Water (PBDW) was added to the Nutrient Agar A slant, following incubation. Using a sterile loop, the growth was dislodged from the agar surface. The mixture was collected, transferred to a vessel containing 99.0 mL of PBDW and mixed thoroughly. A total of 10 Nutrient Agar B plates were inoculated, per test organism, using 200 µL of culture, spreading the inoculum to create a lawn of growth. The plates were incubated at 35-37°C for 24±2 hours. Following incubation, 5.0 mL of Phosphate Buffered Saline + 0.1% Tween 80 was added to each plate. Using a sterile rod/plate spreader, the culture was gently dislodged from the agar surface avoiding disrupting the agar. The culture was collected, combined, and then mixed thoroughly. The collected culture was filtered through sterile Whatman #2 filter paper using a vacuum source. For testing performed on 10/10/16 and 12/6/16, the *Escherichia coli* culture suspension and the *Staphylococcus aureus* culture suspension, respectively, were adjusted using phosphate buffer dilution water to target 1 x 10^9 to 1 x 10^{10} CFU/mL (9-10 logs/mL). For testing performed on 10/10/16, no adjustment was made to the *Staphylococcus aureus* culture suspension. A spectrophotometric analysis was performed using a wavelength of 620 nm. For testing performed on 10/10/16, the final absorbance value was 1.392 for *Staphylococcus aureus* (ATCC 6538) and 1.405 for *Escherichia coli* (ATCC 11229). For testing performed on 12/6/16, the final absorbance value was 1.334 for *Staphylococcus aureus* (ATCC 6538)

Exposure Conditions

Each flask containing the test substance was whirled stopping just before the suspension was added, creating enough residual motion of liquid to prevent pooling of the suspension at the point of contact with test substance. A 1.00 mL aliquot of culture was added midway between the center and edge of the surface with the tip of the pipette slightly immersed in the test solution. Touching the neck or side of the flasks was avoided. Each flask was swirled to thoroughly mix the contents and was exposed for the 30 second exposure time at the exposure temperature 25±1°C (25.0°C).

Test System Recovery

Following exposure, 1.00 mL of the inoculated test substance was transferred to 9 mL of neutralizer. The neutralized material was vortex mixed. The neutralized contents corresponded to the 10^{-1} dilution. Four 1.00 mL and four 0.100 mL aliquots of the neutralized material were spread-plated onto the subculture agar medium.

Incubation and Observation

All subculture plates were incubated for 24-30 hours at 35-37°C. Following incubation, the subculture plates were visually examined for growth.

STUDY CONTROLS

Study controls were performed the same on each test date, unless otherwise noted.

Purity Control
A "streak plate for isolation" was performed on each organism culture and following incubation examined in order to confirm the presence of a pure culture. The acceptance criterion for this study control is a pure culture demonstrating colony morphology typical of the test organism.

Neutralizer Sterility Control
Concurrent with testing, the neutralizer used in testing was evaluated for sterility. A representative sample of neutralizer (1.00 mL), was plated onto the subculture medium as in the test. The plate was incubated and visually examined. The acceptance criterion for this study control is a lack of growth.

PBDW Sterility Control
Concurrent with testing, the PBDW used in testing was evaluated for sterility. A representative sample of PBDW (1.00 mL), was plated onto the subculture medium as in the test. The plate was incubated and visually examined. The acceptance criterion for this study control is a lack of growth.

Test Substance Sterility Control
A representative sample of prepared test substance (1.00 mL), per lot used in testing, was plated onto the subculture agar medium as in the test. Each plate was incubated and visually examined. This control is for informational purposes and therefore has no acceptance criterion.

Numbers Control
A 99.0 mL aliquot of PBDW was transferred to a sterile 250-300 mL Erlenmeyer flask, per test organism. Each flask was equilibrated in a water bath at 25.0°C for ≥10 minutes. Each flask was whirled and 1.00 mL of culture was added as in the test procedure. Each flask was swirled to thoroughly mix the contents. Within approximately 30 seconds, 1.00 mL of the contents was transferred to 9 mL of neutralizer. The neutralized contents correspond to the 10^{-1} dilution. Ten-fold serial dilutions were prepared to 10^{-6}. Four 1.00 mL and four 0.100 mL aliquots of the 10^{-6} dilution were plated onto the subculture agar medium as in the test. This resulted in the 10^{-6} and 10^{-7} dilutions, respectively. The plates were incubated. The acceptance criterion for this control is a minimum value of 7.0 $\log_{10}$.

Neutralization Confirmation Control
The following neutralization confirmation control was performed concurrent with testing. Each prepared test culture was diluted to target $1x10^4$ – $1x10^5$ CFU/mL (to target a result of 10-100 CFU plated in each control run). Multiple organism dilutions were prepared.

Test Culture Titer (TCT)
A 0.100 mL aliquot of diluted test organism was added to 10.0 mL of PBDW and was vortex mixed. The mixture was held for a minimum of 2 minutes and duplicate 0.100 mL aliquots were spread plated as in the test. The acceptance criterion for this study control is growth.

Neutralization Confirmation Control Treatment (NCT)
A 1.00 mL aliquot of test substance, per lot, was added to 9 mL of neutralizer and was vortex mixed. Within approximately 30 seconds, 0.100 mL of diluted test organism was added to the neutralized contents and was vortex mixed. The mixture was held for a minimum of 2 minutes and duplicate 0.100 mL aliquots were spread plated as in the test. The acceptance criterion for this study control is growth within 1 $\log_{10}$ of the test culture titer (TCT).

Neutralizer Toxicity Treatment (NTT)
A 0.100 mL aliquot of diluted test organism was added to 10.0 mL of neutralizer and was vortex mixed. The mixture was held for a minimum of 2 minutes and duplicate 0.100 mL aliquots were spread plated as in the test. The acceptance criterion for this study control is growth within 1 $\log_{10}$ of the test culture titer (TCT).

STUDY ACCEPTANCE CRITERIA

Test Substance Performance Criteria
The efficacy performance requirements for food contact sanitizer label claims state that a product must show a minimum 5 $\log_{10}$ reduction of the test organism as compared to the numbers control.

Control Acceptance Criteria
The study controls must perform according to the criteria detailed in the study controls description section.

PROTOCOL CHANGES

Protocol Amendments:
No protocol amendments were required for this study.

Protocol Deviations:
No protocol deviations occurred during this study.

DATA ANALYSIS

Calculations

The CFU/mL for the test sample and numbers control was determined using counts of 0-300.

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

where 10^{-x} and 10^{-y} were the dilutions plated. In the test procedure, these dilutions corresponded to 10^{-1} and 10^{-2} and in the numbers control; these dilutions correspond to 10^{-6} and 10^{-7} for the 1 mL and 0.1 mL plates, respectively.

Log_{10} Reduction =
Log_{10} (CFU/mL in the numbers control) – Log (CFU/mL in the test sample)

Log_{10} Difference in the Neutralization Confirmation Control =
Log_{10} (Average CFU in TCT) – Log_{10} (Average CFU in NCT or NTT)

An appropriate dilution was used to determine the log_{10} difference in the neutralization confirmation control. A value of <1 was used when no survivors were found in both test dilutions.

Statistical Analysis
None used.

STUDY RETENTION

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

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

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

REFERENCES

1. Association of Official Analytical Chemists (AOAC) Official Method 960.09, Germicidal and Detergent Sanitizing Action of Disinfectants Method. In Official Methods of Analysis of the AOAC, 2013 Edition.
2. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, OCSPP 810.2000: General Considerations for Uses of Antimicrobial Agents, September 4, 2012.
3. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, OCSPP 810.2300: Sanitizers for Use on Hard Surfaces- Efficacy Data Recommendations, September 4, 2012.

RESULTS

For Control and Neutralization Results, see Tables 1-3.
All data measurements/controls including the culture purity, neutralizer sterility, Phosphate Buffer Dilution Water (PBDW) sterility, neutralization confirmation and numbers controls were within acceptance criteria.

For Test Results, see Table 4.

ANALYSIS

D7 (Part 1 Lot 16-353, Part 2 Lot 16-450, Part 3 Lot 18840; Part 1 Lot 16-354, Part 2 Lot 16-451, Part 3 Lot 18840 and Part 1 Lot 16-355, Part 2 Lot 16-452, Part 3 Lot 18840), diluted equal parts Part 1 and Part 2 and 2% Part 3 defined as 49 oz Part 1 + 49 oz Part 2 + 2 oz Part 3, demonstrated a >7.70 Log_{10}, >7.70 Log_{10} and >7.70 Log_{10} reduction of *Escherichia coli* (ATCC 11229), respectively, following a 30 second exposure time at 25±1°C (25.0°C).

D7 (Part 1 Lot 16-353, Part 2 Lot 16-450, Part 3 Lot 18840; Part 1 Lot 16-354, Part 2 Lot 16-451, Part 3 Lot 18840 and Part 1 Lot 16-355, Part 2 Lot 16-452, Part 3 Lot 18840), diluted equal parts Part 1 and Part 2 and 2% Part 3 defined as 49 oz Part 1 + 49 oz Part 2 + 2 oz Part 3, demonstrated a >7.34 Log_{10}, >7.68 Log_{10} and >7.68 Log_{10} reduction of *Staphylococcus aureus* (ATCC 6538), respectively, following a 30 second exposure time at 25±1°C (25.0°C).

CONCLUSION

Under the conditions of this investigation, D7, diluted equal parts Part 1 and Part 2 and 2% Part 3 defined as 49 oz Part 1 + 49 oz Part 2 + 2 oz Part 3, demonstrated efficacy against *Escherichia coli* as required by the U.S. EPA following a 30 second exposure time at 25±1°C (25.0°C).

Under the conditions of this investigation, D7, diluted equal parts Part 1 and Part 2 and 2% Part 3 defined as 49 oz Part 1 + 49 oz Part 2 + 2 oz Part 3, demonstrated efficacy against *Staphylococcus aureus* as required by the U.S. EPA following a 30 second exposure time at 25±1°C (25.0°C).

In the opinion of the Study Director, there were no circumstances that may have adversely affected the quality or integrity of the data.

TABLE 1: CONTROL RESULTS

The following results from controls confirmed study validity:

Test Date: 10/10/16			
Type of Control		**Results**	
		***Escherichia coli* (ATCC 11229)**	***Staphylococcus aureus* (ATCC 6538)**
Purity Control		Pure	Pure
Neutralizer Sterility Control		No Growth	
Test Substance Sterility Control	D7 Part 1 Lot 16-353, Part 2 Lot 16-450, Part 3 Lot 18840	No Growth	
	D7 Part 1 Lot 16-354, Part 2 Lot 16-451, Part 3 Lot 18840	No Growth	
	D7 Part 1 Lot 16-355, Part 2 Lot 16-452, Part 3 Lot 18840	No Growth	
PBDW Sterility Control		No Growth	

Test Date: 12/6/16		
Type of Control		**Results**
		***Staphylococcus aureus* (ATCC 6538)**
Purity Control		Pure
Neutralizer Sterility Control		No Growth
Test Substance Sterility Control	D7 Part 1 Lot 16-353, Part 2 Lot 16-450, Part 3 Lot 18840	No Growth
PBDW Sterility Control		No Growth

PBDW = Phosphate Buffer Dilution Water

TABLE 2: NUMBERS CONTROL RESULTS

Test Date: 10/10/16			
Test Organism	**Survivors (CFU) Volume plated**		**Population Control Result**
	1.00 mL (10^{-6})	**0.100 mL (10^{-7})**	
Escherichia coli (ATCC 11229)	56, 44, 39, 40	12, 8, 11, 8	5.0×10^7 CFU/mL (7.70 Log_{10})
Staphylococcus aureus (ATCC 6538)	53, 40, 46, 46	7, 6, 6, 7	4.8×10^7 CFU/mL (7.68 Log_{10})
Test Date: 12/6/16			
Test Organism	**Survivors (CFU) Volume plated**		**Population Control Result**
	1.00 mL (10^{-6})	**0.100 mL (10^{-7})**	
Staphylococcus aureus (ATCC 6538)	25, 16, 27, 18	3, 1, 0, 2	2.2×10^7 CFU/mL (7.34 Log_{10})

CFU = Colony Forming Units

TABLE 3: NEUTRALIZATION CONFIRMATION CONTROL RESULTS

Test Date: 10/10/16					
Test Organism: *Escherichia coli* (ATCC 11229)					
Control Identity or Test Substance Identity	**Dilution**	**Survivors (CFU)**	**Test Culture Titer (TCT)**	**Log_{10} Difference**	**Pass/ Fail (± 1 Log_{10})**
Neutralizer Toxicity Treatment (NTT)	10^{-4}	T, T	T, T	-0.11	Pass
	10^{-5}	47, 46	33, 39		
D7 Part 1 Lot 16-353, Part 2 Lot 16-450, Part 3 Lot 18840 for NCT	10^{-4}	82, 82	T, T	0.33	Pass
	10^{-5}	16, 17	33, 39		
D7 Part 1 Lot 16-354, Part 2 Lot 16-451, Part 3 Lot 18840 for NCT	10^{-4}	138, 128	T, T	0.52	Pass
	10^{-5}	13, 9	33, 39		
D7 Part 1 Lot 16-355, Part 2 Lot 16-452, Part 3 Lot 18840 for NCT	10^{-4}	76, 94	T, T	0.52	Pass
	10^{-5}	11, 11	33, 39		

NCT = Neutralization Confirmation Control Treatment
CFU = Colony Forming Units
T = Too Numerous To Count (>300 colonies)

TABLE 3: NEUTRALIZATION CONFIRMATION CONTROL RESULTS - CONTINUED

Control Identity or Test Substance Identity	Dilution	Survivors (CFU)	Test Culture Titer (TCT)	Log_{10} Difference	Pass/ Fail (± 1 Log_{10})
Test Date: 10/10/16					
Test Organism: *Staphylococcus aureus* (ATCC 6538)					
Neutralizer Toxicity Treatment (NTT)	10^{-4}	T, T	144, 140	-0.24	Pass
	10^{-5}	53, 50	27, 33		
D7 Part 1 Lot 16-354, Part 2 Lot 16-451, Part 3 Lot 18840 for NCT	10^{-4}	11, 8	144, 140	1.00	Pass
	10^{-5}	3, 3	27, 33		
D7 Part 1 Lot 16-355, Part 2 Lot 16-452, Part 3 Lot 18840 for NCT	10^{-4}	10, 8	144, 140	1.00	Pass
	10^{-5}	1, 4	27, 33		
Test Date: 12/6/16					
Test Organism: *Staphylococcus aureus* (ATCC 6538)					
Neutralizer Toxicity Treatment (NTT)	10^{-4}	162, 250	178, 192	-0.09	Pass
	10^{-5}	29, 25	22, 21		
D7 Part 1 Lot 16-353, Part 2 Lot 16-450, Part 3 Lot 18840 for NCT	10^{-4}	45, 82	178, 192	0.49	Pass
	10^{-5}	5, 8	22, 21		

NCT = Neutralization Confirmation Control Treatment
CFU = Colony Forming Units
T = Too Numerous To Count (>300 colonies)

TABLE 4: TEST RESULTS

Test Substance	Survivors (CFU) Volume plated 1.00 mL (10^{-1})	Survivors (CFU) Volume plated 0.100 mL (10^{-2})	Test Results	Log_{10} Reduction
Test Date: 10/10/16				
Test Organism: *Escherichia coli* (ATCC 11229)				
D7 Part 1 Lot 16-353, Part 2 Lot 16-450, Part 3 Lot 18840	0, 0, 0, 0	0, 0, 0, 0	<1 CFU/mL (<0.00 Log_{10})	>7.70
D7 Part 1 Lot 16-354, Part 2 Lot 16-451, Part 3 Lot 18840	0, 0, 0, 0	0, 0, 0, 0	<1 CFU/mL (<0.00 Log_{10})	>7.70
D7 Part 1 Lot 16-355, Part 2 Lot 16-452, Part 3 Lot 18840	0, 0, 0, 0	0, 0, 0, 0	<1 CFU/mL (<0.00 Log_{10})	>7.70
Test Organism: *Staphylococcus aureus* (ATCC 6538)				
D7 Part 1 Lot 16-354, Part 2 Lot 16-451, Part 3 Lot 18840	0, 0, 0, 0	0, 0, 0, 0	<1 CFU/mL (<0.00 Log_{10})	>7.68
D7 Part 1 Lot 16-355, Part 2 Lot 16-452, Part 3 Lot 18840	0, 0, 0, 0	0, 0, 0, 0	<1 CFU/mL (<0.00 Log_{10})	>7.68
Test Date: 12/6/16				
Test Organism: *Staphylococcus aureus* (ATCC 6538)				
D7 Part 1 Lot 16-353, Part 2 Lot 16-450, Part 3 Lot 18840	0, 0, 0, 0	0, 0, 0, 0	<1 CFU/mL (<0.00 Log_{10})	>7.34

CFU = Colony Forming Units
A value of <1 was used in place of zero for calculation purposes.

ATTACHMENT I: INVALID DATA – TEST DATE 10/10/16

NOTE: Due to a neutralization confirmation control failure, testing of test substance D7 Part 1 Lot 16-353, Part 2 Lot 16-450 and Part 3 Lot 18840 against Staphylococcus aureus was repeated.

Date Performed: October 10, 2016
Test Substance: D7 Part 1 Lot 16-353, Part 2 Lot 16-450 and Part 3 Lot 18840
Dilution: Equal parts Part 1 and Part 2 and 2% Part 3 defined as 49 oz Part 1 + 49 oz Part 2 + 2 oz Part 3
Test Organism: *Staphylococcus aureus* (ATCC 6538)
Growth Medium: Nutrient Agar A & B
Neutralizer: D/E Neutralizing Broth + 0.01% Catalase
Organic Soil Load: No organic soil load required
Exposure Time: 30 seconds

NEUTRALIZATION CONFIRMATION CONTROL RESULTS

Test Organism: Staphylococcus aureus (ATCC 6538)					
Control Identity or Test Substance Identity	**Dilution**	**Survivors (CFU)**	**Test Culture Titer (TCT)**	**Log_{10} Difference**	**Pass/ Fail (± 1 Log_{10})**
D7 Part 1 Lot 16-353, Part 2 Lot 16-450, Part 3 Lot 18840 for NCT	10^{-4}	10, 9	144, 140	1.18	Fail
	10^{-5}	1, 2	27, 33		

NCT = Neutralization Confirmation Control Treatment
CFU = Colony Forming Units

TEST RESULTS

Test Organism: Staphylococcus aureus (ATCC 6538)				
Test Substance	**Survivors (CFU)**		**Test Results**	**Log_{10} Reduction**
	Volume plated			
	1.00 mL (10^{-1})	**0.100 mL (10^{-2})**		
D7 Part 1 Lot 16-353, Part 2 Lot 16-450, Part 3 Lot 18840	0, 0, 0, 0	0, 0, 0, 0	<1 CFU/mL (<0.00 Log_{10})	>7.68

CFU = Colony Forming Units
A value of <1 was used in place of zero for calculation purposes.

ATTACHMENT II: INVALID DATA – TEST DATE 11/2/16

NOTE: Due to a neutralization confirmation control failure, testing of test substance D7 Part 1 Lot 16-353, Part 2 Lot 16-450 and Part 3 Lot 18840 against Staphylococcus aureus was repeated.

Date Performed:	November 2, 2016
Test Substance:	D7 Part 1 Lot 16-353, Part 2 Lot 16-450 and Part 3 Lot 18840
Dilution:	Equal parts Part 1 and Part 2 and 2% Part 3 defined as 49 oz Part 1 + 49 oz Part 2 + 2 oz Part 3
Test Organism:	*Staphylococcus aureus* (ATCC 6538)
Growth Medium:	Nutrient Agar A & B
Neutralizer:	D/E Neutralizing Broth + 0.07% Lecithin + 0.5% Tween 80+ 0.1% Sodium Thiosulfate + 0.01% Catalase
Organic Soil Load:	No organic soil load required
Exposure Time:	30 seconds

CONTROL RESULTS

Type of Control		Results: *Staphylococcus aureus* (ATCC 6538)
Purity Control		Pure
Neutralizer Sterility Control		No Growth
Test Substance Sterility Control	D7 Part 1 Lot 16-353, Part 2 Lot 16-450 and Part 3 Lot 18840	No Growth
PBDW Sterility Control		No Growth

PBDW = Phosphate Buffer Dilution Water

NUMBERS CONTROL RESULTS

Test Organism	Survivors (CFU)		Population Control Result
	Volume plated		
	1.00 mL (10^{-6})	0.100 mL (10^{-7})	
Staphylococcus aureus (ATCC 6538)	T, T, T, T	316, 354, 272, 264	3.02×10^9 CFU/mL (9.48 Log_{10})

CFU = Colony Forming Units

NEUTRALIZATION CONFIRMATION CONTROL RESULTS

Test Organism: *Staphylococcus aureus* (ATCC 6538)			
Control Identity or Test Substance Identity	**Dilution**	**Survivors (CFU)**	**Test Culture Titer (TCT)**
Neutralizer Toxicity Treatment (NTT)	10^{-3}	T, T	T, T
	10^{-4}	T, T	236, 187
	10^{-5}	40, 49	38, 26
D7 Part 1 Lot 16-353, Part 2 Lot 16-450, Part 3 Lot 18840 for NCT	10^{-3}	0, 0	T, T
	10^{-4}	0, 0	236, 187
	10^{-5}	0, 0	38, 26

NCT = Neutralization Confirmation Control Treatment
CFU = Colony Forming Units
T = Too Numerous To Count (>300 colonies)

TEST RESULTS

Test Organism: *Staphylococcus aureus* (ATCC 6538)		
Test Substance	**Survivors (CFU)**	
	Volume plated	
	1.00 mL (10^{-1})	**0.100 mL (10^{-2})**
D7 Part 1 Lot 16-353, Part 2 Lot 16-450, Part 3 Lot 18840	0, 0, 0, 0	0, 0, 0, 0

CFU = Colony Forming Units

ATTACHMENT III: TEST SUBSTANCE CERTIFICATE OF ANALYSIS – Part 1

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

8/30/2016

Certificate of Analysis Decon7 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[attached]. Expiration date to all product is 08/23/2017.

batch number	%wt. Alkyl Dimethylbenzyl Ammonium Chloride	LCL	UCL
16-353	3.05	3.04	3.36
16-354	3.04	3.04	3.36
16-355	3.06	3.04	3.36

EXACT COPY
INITIALS SLA DATE 12-15-16

ATTACHMENT IV: TEST SUBSTANCE CERTIFICATE OF ANALYSIS – Part 2

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

8/30/2016

Certificate of Analysis Decon7 Part 2

Decon7Part 2 is assayed for %wt.H_2O_2 using method BCQCSP – 6.44. Expiration date to all product is 08/19/2017.

batch number	%wt. H_2O_2	LCL	UCL
16-450	7.53	7.51	8.3
16-451	7.55	7.51	8.3
16-452	7.542	7.51	8.3

EXACT COPY
INITIALS JLH DATE 12-15-16

(For Laboratory Use Only) A21809
Accuratus Lab Services Project # KJP9-23-16
Test Substance Tracking # TS090216.DSS01.2 NDK 9/7/16
TS120616.DSS01 JLH 12-6-16

ACCURATUS LAB SERVICES

PROTOCOL

Germicidal and Detergent Sanitizing Action of Disinfectants

Test Organism(s):

Escherichia coli (ATCC 11229)
Staphylococcus aureus (ATCC 6538)

PROTOCOL NUMBER

DSS01072816.GDST

PREPARED FOR/SPONSOR

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

PREPARED BY/TESTING FACILITY

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

DATE

July 28, 2016

Revised August 3, 2016

EXACT COPY
INITIALS JLH DATE 12-15-16

Germicidal and Detergent Sanitizing Action of Disinfectants

PURPOSE
The purpose of this assay is to determine the efficacy of a product to sanitize pre-cleaned, nonporous food contact surfaces using the AOAC Germicidal and Detergent Sanitizing Action of Disinfectants method. This method is in compliance with the requirements of and may be submitted to, one or more of the following agencies as indicated by the Sponsor: U.S. Environmental Protection Agency (EPA) and Health Canada.

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

SCHEDULING AND DISCLAIMER OF WARRANTY
Experimental start dates are generally scheduled on a first-come/first-serve basis once Accuratus Lab Services receives the Sponsor approved/completed protocol, signed fee schedule and corresponding test substance(s). Based on all required materials being received at this time, the proposed experimental start date is August 12, 2016. Verbal results may be given upon completion of the study with a written report to follow on the proposed completion date of September 9, 2016. To expedite scheduling, please be sure all required paperwork and test substance documentation is complete/accurate upon arrival at Accuratus Lab Services.

If a test must be repeated, or a portion of it, due to failure by Accuratus Lab Services to adhere to specified procedures, it will be repeated free of charge. If a test must be repeated, or a portion of it, due to failure of internal controls, it will be repeated free of charge. "Methods Development" fees shall be assessed, however, if the test substance and/or test system require modifications due to complexity and difficulty of testing. If the Sponsor requests a repeat test, they will be charged for an additional test. Neither the name of Accuratus Lab Services nor any of its employees are to be used in advertising or other promotion without written consent from Accuratus Lab Services. The Sponsor is responsible for any rejection of the final report by the regulating agencies concerning report format, pagination, etc. To prevent rejection, Sponsor should carefully review the Accuratus Lab Services final report and notify Accuratus Lab Services of any perceived deficiencies in these areas before submission of the report to the regulatory agency. Accuratus Lab Services will make reasonable changes deemed necessary by the Sponsor, without altering the technical data.

JUSTIFICATION FOR SELECTION OF THE TEST SYSTEM
Regulatory agencies require that a specific claim for a sanitizer intended for use on food contact surfaces be supported by appropriate scientific data demonstrating the efficacy of the product against the claimed test organism. This is accomplished by treating the target organism with the test substance under conditions which simulate as closely as possible, in the laboratory, the actual conditions under which the sanitizer is designed to be used. For sanitizer products intended for use on food contact surfaces, a suspension method is used in the generation of the supporting data. The experimental design in this protocol meets these requirements. The test system to be used in this study will follow the AOAC approved method for the determination of the Germicidal and Detergent Sanitizing Activity of Disinfectants. A consistent subculture agar medium will be used to recover survivors in the test, population control and neutralization confirmation control to eliminate recovery variability that may be associated with multiple agar media. An agar medium with additional neutralizers may be used to further assist with neutralization. Health Canada requires the product be a broad spectrum or hospital grade disinfectant before sanitizer claims may be made.

TEST PRINCIPLE

A suspension of test organism cells is exposed to the test substance for a specified exposure time. After exposure, an aliquot of the exposed suspension is transferred to vessels containing neutralizer and assayed for survivors. Appropriate numbers, culture purity, sterility, and neutralization confirmation controls are performed. The current revision of Standard Operating Procedure CGT-0024 reflects the methods which shall be used in this study.

TEST METHOD

Table 1:

Test Organism	Designation #	Growth Medium	Incubation Parameters	Recommended optical density target at 620nm
Escherichia coli	11229	Nutrient Agar A & B	35-37°C, aerobic	1.3-1.5
Staphylococcus aureus	6538	Nutrient Agar A & B	35-37°C, aerobic	1.3-1.5

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

Suggested Subculture Agar/Plating Method:
Tryptone Glucose Extract Agar / Pour-plating OR
Tryptic Soy Agar + 5% Sheep's Blood / Spread-plating

Preparation of Test Organism

For *Staphylococcus aureus* (ATCC 6538) and/or *Escherichia coli* (ATCC 11229), streak a loopful of a thawed cryovial of stock organism broth culture to a Nutrient Agar A slant medium. Incubate at 35-37°C for 24±2 hours.

For the final test culture, add 5 mL of Phosphate Buffer Dilution Water (PBDW) to the Nutrient Agar A slant, following incubation. **Using a sterile loop,** dislodge growth from the agar surface. Collect the mixture and transfer to a vessel containing 99 mL of PBDW and mix thoroughly. Inoculate a minimum of 5 Nutrient Agar B plates using 200 µL of culture, spreading the inoculum to create a lawn of growth. Incubate the plates at 35-37°C for 24±2 hours.

Following incubation, add a minimum of 5 mL of Phosphate Buffered Saline + 0.1% Tween 80 to each plate. Using a sterile rod/plate spreader, gently dislodge the culture from the agar surface. Avoid disrupting the agar. Collect and combine the culture, then mix thoroughly. Filter the collected culture through sterile Whatman #2 filter paper using a vacuum source. Standardize the culture, as necessary, to target 1×10^9 to 1×10^{10} CFU/mL (9-10 logs/mL). The use of a spectrophotometer is recommended. Applicable culture dilutions will be prepared using PBDW.

An organic soil load may be added to the test culture per Sponsor's request.

Preparation of Test Substance

The test substance(s) to be assayed will be used as directed by the Sponsor. If a dilution of the test substance is requested by the Sponsor, the diluted test substance(s) shall be used within three hours of preparation. Transfer 99 mL of test substance to a sterile 250 – 300 mL Erlenmeyer flask per test organism. Place the flask into a water bath at the Sponsor specified exposure temperature and equilibrate for ≥10 minutes.

Exposure Conditions

The flask containing the test substance will be whirled stopping just before the suspension is added, creating enough residual motion of liquid to prevent pooling of the suspension at the point of contact with test substance. A 1.0 mL aliquot of culture will be added midway between the center and edge of the surface with the tip of the pipette slightly immersed in the test solution. Touching the neck or side of the flasks will be avoided. Swirl the flask to thoroughly mix the contents. Allow the solution to expose for the exposure time.

Test System Recovery
Following exposure, 1.0 mL of the inoculated test substance will be transferred to 9 mL of neutralizer. The neutralized material will be vortex mixed. The neutralized contents correspond to the 10^{-1} dilution. Four 1.0 mL and four 0.1 mL aliquots of the neutralized material will be transferred to individual sterile Petri dishes and pour-plated using an appropriate subculture agar medium. Alternately, the aliquots may be spread-plated onto an appropriate subculture agar medium.

Incubation and Observation
All subculture plates are incubated under the conditions listed in table 1 for 24-30 hours. If applicable, pour-plates will be allowed to solidify and will be inverted prior to incubation. Additional incubation may be followed if colonial growth is difficult to visually detect. Subcultures may be stored at 2-8°C for up to 3 days prior to reading. Following incubation (or incubation and storage), the subculture plates will be visually examined for growth.

Representative subculture plates showing growth will be stained and/or biochemically assayed to confirm or rule out the presence of the test organism. Additional subculturing may be performed, if necessary.

STUDY CONTROLS

Purity Control
A "streak plate for isolation" will be performed on the organism culture and following incubation examined in order to confirm the presence of a pure culture. The acceptance criterion for this study control is a pure culture demonstrating colony morphology typical of the test organism.

Organic Soil Sterility Control
Prior to or concurrent with testing and if applicable, the serum used for the organic soil load will be cultured, incubated, and visually examined for lack of growth. The acceptance criterion for this study control is lack of growth.

Neutralizer Sterility Control
Prior to or concurrent with testing, the neutralizer used in testing will be evaluated for sterility. A representative sample of neutralizer (1.0 mL), per lot of neutralizer used in testing, will be plated onto the subculture agar medium as in the test. The plate will be incubated and visually examined. The acceptance criterion for this study control is a lack of growth.

Test Substance Diluent Sterility Control
Prior to or concurrent with testing, the test substance diluent used in testing will be evaluated for sterility, if applicable. A representative sample of test substance diluent (1.0 mL), per lot used in testing, will be plated onto the subculture agar medium as in the test. The plate will be incubated and visually examined. The acceptance criterion for this study control is a lack of growth.

PBDW Sterility Control
Prior to or concurrent with testing, the PBDW used in testing will be evaluated for sterility. A representative sample of PBDW (1.0 mL), per lot used in testing, will be plated onto the subculture agar medium as in the test. The plate will be incubated and visually examined. The acceptance criterion for this study control is a lack of growth.

Test Substance Sterility Control
A representative sample of prepared test substance (1.0 mL), per sample or lot used in testing, will be plated onto the subculture agar medium as in the test. The plate will be incubated and visually examined. This control is for informational purposes and therefore has no acceptance criterion.

Numbers Control
Transfer 99 mL of PBDW to a sterile 250-300 mL Erlenmeyer flask. Equilibrate the flask in a water bath to the specified exposure temperature for ≥10 minutes.

Whirl the flask and add 1.0 mL of culture as in the test procedure. Swirl the flask to thoroughly mix the contents.

Within approximately 30 seconds, transfer 1 mL of the contents to 9 mL of neutralizer. **The neutralized contents correspond to the 10^{-1} dilution.**

Prepare ten-fold serial dilutions to 10^{-6}. Plate four 1.0 mL and four 0.1 mL aliquots of the 10^{-6} dilution onto the subculture agar medium as in the test. (This results in the 10^{-6} and 10^{-7} dilutions, respectively.) Incubate the plates. The acceptance criterion for this control is a minimum value of 7.0 $\log_{10}$.

Neutralization Confirmation Control
The following neutralization confirmation control will be performed prior to testing or concurrent with testing. Only the most concentrated test substance dilution needs to be evaluated. Serially dilute the prepared test culture to target $1x10^4 - 1x10^5$ CFU/mL (to target a result of 10-100 CFU plated in each control run). Multiple organism dilutions may be prepared. *(Typically the 10^{-4} and 10^{-5} dilutions will provide a culture in range. Alternate dilutions may be used where appropriate.)* If all the organism dilution(s) used in this control fail to provide adequate numbers which coincides in a failure to meet the acceptance criterion for this study control, the control may be repeated in its entirety.

Test Culture Titer (TCT)
Add 0.1 mL of diluted test organism to 10 mL of PBDW and vortex mix. Hold the mixture for a minimum of 2 minutes and spread plate or pour plate duplicate 0.1 mL aliquots as in the test. The acceptance criterion for this study control is growth.

Neutralization Confirmation Control Treatment (NCT)
Add 1.0 mL of test substance to 9 mL of neutralizer and vortex mix. Within approximately 30 seconds, add 0.1 mL of diluted test organism to the neutralized contents and vortex mix. Hold the mixture for a minimum of 2 minutes and spread plate or pour plate duplicate 0.1 mL aliquots as in the test. The acceptance criterion for this study control is growth within 1 $\log_{10}$ of the test culture titer (TCT).

Neutralizer Toxicity Treatment (NTT)
Add 0.1 mL of diluted test organism to 10 mL of neutralizer and vortex mix. Hold the mixture for a minimum of 2 minutes and spread plate or pour plate duplicate 0.1 mL aliquots as in the test. The acceptance criterion for this study control is growth within 1 $\log_{10}$ of the test culture titer (TCT).

PROCEDURE FOR IDENTIFICATION OF THE TEST SYSTEM

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

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

METHOD FOR CONTROL OF BIAS: NA

STUDY ACCEPTANCE CRITERIA

Test Substance Performance Criteria
The efficacy performance requirements for food contact sanitizer label claims state that a product must show a minimum 5 $\log_{10}$ reduction of the test organism as compared to the numbers control.

Control Acceptance Criteria
The study controls must perform according to the criteria detailed in the study controls description section. If any of the control acceptance criteria are not met, the test may be repeated under the current protocol. If the numbers control exceeds an average $\log_{10}$ value of 8.0 and the test substance fails to meet the acceptance criteria, the Sponsor may invalidate the study and repeat testing.

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

REPORT

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

PROTOCOL CHANGES

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

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

TEST SUBSTANCE RETENTION

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

RECORD RETENTION

Study Specific Documents

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

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

Facility Specific Documents

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

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

REFERENCES

1. Association of Official Analytical Chemists (AOAC) Official Method 960.09, Germicidal and Detergent Sanitizing Action of Disinfectants Method. In Official Methods of Analysis of the AOAC, 2013 Edition.
2. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, OCSPP 810.2000: General Considerations for Uses of Antimicrobial Agents, September 4, 2012.
3. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, OCSPP 810.2300: Sanitizers for Use on Hard Surfaces- Efficacy Data Recommendations, September 4, 2012.
4. Health Canada, January, 2014. Guidance Document – Safety and Efficacy Requirements for Hard Surface Disinfectant Drugs.
5. Health Canada, January, 2014. Guidance Document - Disinfectant Drugs.

DATA ANALYSIS

Calculations

Determine the CFU/mL for the test sample and numbers control using counts of 0-300.

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

where 10^{-x} and 10^{-y} are the dilutions plated. In the test procedure, these dilutions correspond to 10^{-1} and 10^{-2} and in the numbers control, these dilutions correspond to 10^{-6} and 10^{-7} for the 1 mL and 0.1 mL plates, respectively. Where no survivors are found in both test dilutions, the final value will be expressed as <1 CFU/mL. If both dilutions are TNTC, substitute >300 at the highest (most dilute) dilution.

Log_{10} Reduction = Log_{10} (CFU/mL in the numbers control) – Log (CFU/mL in the test sample)

Log_{10} Difference in the Neutralization Confirmation Control = Log_{10} (Average CFU in TCT) – Log_{10} (Average CFU in NCT or NTT)
An appropriate dilution will be used to determine the $\log_{10}$ difference in the neutralization confirmation control.

Statistical Analysis:
None used

STUDY INFORMATION

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

Test Substance (Name & Batch Numbers) exactly as it should appear on final report:
D7 Part 1 Lot 16-353, Part 2 Lot 16-450, Part 3 Lot 18840
D7 Part 1 Lot 16-354, Part 2 Lot 16-451, Part 3 Lot 18840
D7 Part 1 Lot 16-355, Part 2 Lot 16-452, Part 3 Lot 18840
Testing at the lower certified limit (LCL) is required for registration, no aged batch is necessary.

Product Description:
- ☑ Quaternary ammonia
- ☑ Peroxide
- ☐ Peracetic acid
- ☐ Sodium hypochlorite
- ☐ Iodophor
- ☑ Other: Diacetin

Approximate Test Substance Active Concentration (upon submission to Accuratus Lab Services):
3% Quat, 7.9% Peroxide, 99% Diacetin (Part 3)
(This value is used for neutralization planning only. This value is not intended to represent characterization values.)

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

Storage Conditions:
- ☑ Room Temperature
- ☐ 2-8°C
- ☐ Other:_____

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

Product Preparation
- ☐ No dilution required, Use as received (RTU)
- ☑ *Dilution(s) to be tested:
  Mix equal parts of Part 1 and Part 2. Add 2% Part 3 to solution. Mix solution and allow to stand for 5 minutes. Defined as 49 oz Part 1 + 49 oz Part 2 + 2 oz Part 3.
  - ☐ Deionized Water (Filter or Autoclave Sterilized)
  - ☐ Tap Water (Filter or Autoclave Sterilized) - All tap water is softened; the water hardness for the batch of tap water used will be determined and reported.
  - ☐ AOAC Synthetic Hard Water: _____PPM
  - ☐ Other _____

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

Test Organism(s):
- ☑ *Staphylococcus aureus* (ATCC 6538)
- ☑ *Escherichia coli* (ATCC 11229)

Exposure Time: 30 Seconds

Exposure Temperature:
- ☑ 25 ± 1°C*
- ☐ Other:_____ ±1°C

Organic Soil Load *(generally not required for food contact sanitizer label claims):*
- ☑ No Organic Soil Load Required
- ☐ Minimum 5% Organic Soil Load (Fetal Bovine Serum)
- ☐ Other:_____

TEST SUBSTANCE SHIPMENT STATUS
(This section is for informational purposes only.)
☐ Test Substance is already present at Accuratus Lab Services.
☑ Test Substance has been or will be shipped to Accuratus Lab Services.
Date of expected receipt at Accuratus Lab Services: BY AUGUST 29, 2016
☐ Test Substance to be hand-delivered (must arrive by noon at least one day prior to testing or other arrangements made with the Study director).

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

REGULATORY AGENCY(S) THAT MAY REVIEW DATA
☑ U.S. EPA
☐ Health Canada

PROTOCOL MODIFICATIONS
☑ Approved without modification
☐ Approved with modification

PROTOCOL ATTACHMENTS
Supplemental Information Form Attached - ☐ Yes ☑ No

TEST SUBSTANCE CHARACTERIZATION & STABILITY TESTING
[Verification required per 40 CFR Part 160 Subpart B (160.31(d))].

☐ Characterization/Stability testing is not required (For Non-GLP or Development testing only)

OR

Physical and Chemical Characterization (Identity, purity, strength, solubility, as applicable) of the test lots

☑ **Physical & Chemical Characterization has been or will be completed prior to efficacy testing.**

GLP compliance status of physical & chemical characterization testing:
☐ Testing was or will be performed following 40 CFR Part 160 GLP regulations
① ☑ Characterization has not been or will not be performed following GLP regulations

Check and complete the following that apply:
☑ A Certificate of Analysis (C of A) may be provided for each lot of test substance. If provided, the C of A will be appended to the report.
☐ Testing has been or will be conducted at Accuratus Lab Services under protocol or study #: _____
☐ Test has been or will be conducted by another facility under protocol or study #: _____

☐ **Physical & Chemical Characterization was not or will not be performed prior to efficacy testing.**

Stability Testing of the formulation

☑ **Stability testing has been or will be completed prior to or concurrent with efficacy testing.**

GLP compliance status of stability testing:
(GLP compliance is required by 40 CFR Part 160)
☐ Testing was or will be performed following 40 CFR Part 160 GLP regulations
① ☑ Stability testing has not been or will not be performed following GLP regulations

Check and complete the following that apply:
☐ Testing has been or will be conducted at Accuratus Lab Services under protocol or study #: _____
☐ Test has been or will be conducted by another facility under protocol or study #: _____

☐ **Stability testing was not or will not be performed prior to or concurrent with efficacy testing.**

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

① Added per phone conversation note. JLH 9-16-16

APPROVAL SIGNATURES

SPONSOR:

NAME: Mr. Joe Drake TITLE: President/Founder

SIGNATURE: DATE: 8/3/2016

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

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

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

Accuratus Lab Services:

NAME: Jamie Herzan
Study Director

SIGNATURE: Jamie Herzan DATE: 9-22-16
Study Director