STUDY TITLE
GLP ASTM E1053 Method

Study Identification Number
GLP1858

Protocol Number
P2046

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

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

Test Substance Name: D7 Part 3
Lot Number: 20335

Test Microorganism
Feline calicivirus, Strain F-9, ATCC VR-782 (EPA-Approved Human Norovirus Surrogate)

Data Requirements
U.S. EPA 40 CFR Part 158
U.S. EPA OCSPP 810.2200

Author
Kelli Jo Vagias, B.S.
Study Director

Study Completion Date
19FEB2018

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

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

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

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

Company: _____

Agent/Submitter: _____

Title: _____

Date: _____

Signature: _____

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GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study meets U.S. Environmental Protection Agency's Good Laboratory Practice Standards and requirements for 40 CFR § 158 with the following exception:

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

Study Director

Company: Microchem Laboratory

Name: Kelli Jo Vagias, B.S.

Title: Study Director

Signature: _____ Date: 19FEB2018

Study Sponsor

Company: Decon 7

Name: Brian Narducci

Title: Study Sponsor

Signature: _____ Date: 03/07/2018

Submitter

Company:

Name:

Title:

Signature: _____ Date: _____

QUALITY ASSURANCE STATEMENT

Study Title: GLP ASTM E1053 Method

Study ID#: GLP1858

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	29DEC2017	29DEC2017	03JAN2018
Draft Report	02FEB2018	02FEB2018	05FEB2018
Final Report	19FEB2018	19FEB2018	19FEB2018

Quality Assurance Unit:

Signature: Date: 19FEB2018

Name: Inniiko Lutz, B.S.
Title: Quality Assurance Specialist

PERSONNEL INVOLVED IN THE STUDY

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

Study Director
Name: Kelli Jo Vagias, B.S.
Company: Microchem Laboratory
Title: Study Director

Assisting Personnel
Name: Madhuri Patil, M.S.
Company: Microchem Laboratory
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Name: Donna Marsh
Company: Microchem Laboratory
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Name: Connor Weeks, B.S.
Company: Microchem Laboratory
Title: Technician

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FINAL STUDY REPORT SUMMARY

Study Title
GLP ASTM E1053 Method

Study Identification Number
GLP1858

Protocol Number
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Test Microorganism
Feline calicivirus, Strain F-9, ATCC VR-782

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

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

Study Director
Kelli Jo Vagias, B.S.

Study Completion Date
19FEB2018

Study Objective
To determine, using the ASTM E1053 Virucidal Activity of Chemicals Intended for Disinfection of Inanimate, Nonporous Environmental Surfaces method, the virucidal efficacy of D7 Part 1 (A) + D7 Part 2 (B) + D7 Part 3 diluted to a ratio of (49:49:2) (D7 Part 1 (A) Lots: 17-390 & 17-391, D7 Part 2 (B) Lots: 17-393 & 17-394, and D7 Part 3 Lot: 20335) against Feline calicivirus, Strain F-9, ATCC VR-782 supplemented to a contain 5.0 ± 0.1% (v/v) Fetal Bovine Serum (FBS) at a contact time of 9 minutes & 50 seconds ± 5 seconds.

Study Conclusion in Brief
Test substance D7 Part 1 (A) + D7 Part 2 (B) + D7 Part 3 diluted to a ratio of (49:49:2) (D7 Part 1 (A) Lots: 17-390 & 17-391, D7 Part 2 (B) Lots: 17-393 & 17-394, and D7 Part 3 Lot: 20335) met the U.S. EPA Product Performance Guideline for Disinfectants for Use on Hard Surfaces outlined in OCSPP 810.2200 when tested against Feline calicivirus, Strain F-9, ATCC VR-782 supplemented to contain 5.0 ± 0.1% (v/v) Fetal Bovine Serum (FBS) at a contact time of 9 minutes & 50 seconds ± 5 seconds.

FINAL STUDY REPORT

Important Dates

Study Initiation Date: 27DEC2017
Experimental Start Date: 29DEC2017 / 1700
Experimental End Date: 04JAN2018 / 1524

Test Substance Information

Name: D7 Part 1 (A)

Lot: 17-390
Active Ingredients: Alkyl Dimethylbenzyl Ammonium Chloride
Active Ingredient Concentration: 3.04%
Manufacture Date: 01AUG2017
Date Received: 05SEP2017
Expiration Date: 01AUG2018

Lot: 17-391
Active Ingredients: Alkyl Dimethylbenzyl Ammonium Chloride
Active Ingredient Concentration: 3.06%
Manufacture Date: 01AUG2017
Date Received: 05SEP2017
` Expiration Date: 01AUG2018

Name: D7 Part 2 (B)

Lot: 17-393
Active Ingredients: Hydrogen Peroxide
Active Ingredient Concentration: 7.528%
Manufacture Date: 28JUL2017
Date Received: 05SEP2017
` Expiration Date: 28JUL2018

Lot: 17-394
Active Ingredients: Hydrogen Peroxide
Active Ingredient Concentration: 7.469%
Manufacture Date: 28JUL2017
Date Received: 05SEP2017
` Expiration Date: 28JUL2018

FINAL STUDY REPORT (cont.)

Test Substance Information (cont.)

Name: D7 Part 3

Lot: 20335
Active Ingredients: Diacetain
Active Ingredient Concentration: Not provided by Study Sponsor
Manufacture Date: 14SEP2016
Date Received: 05SEP2017
Expiration Date: 14SEP2018

Form: Liquid, Dilution Required

Storage Conditions: The test substance was stored at ambient (room) temperature under fluorescent lighting or in a cabinet.

FINAL STUDY REPORT (cont.)

Test Parameters

Microorganism:	Feline calicivirus, Strain F-9, ATCC VR-782
Stock Lot Number:	FCV_10NOV2017
Culture Manipulation:	Viral stock was diluted in 1:10 dilutions three times prior to use in testing.
Host Cell Line:	CRFK, ATCC CCL-94
Subculture Passage Number:	200
Test Assay Medium:	Eagle's Minimum Essential Medium (EMEM) supplemented with 2% fetal bovine serum plus antibiotics [100 µg/ml kanamycin sulfate solution and antibiotic-antimycotic solution (100 units/ml penicillin G, 100 µg/ml streptomycin, and 0.25 µg/ml amphotericin B)]
Number of Test Carriers:	4 (2 dried virus films per test substance batch)
Carrier Type:	Glass Petri dishes (100 x 15 mm)
Contact Time:	9 minutes & 50 seconds ± 5 seconds
Test Substance Form:	Liquid; Dilution Required
Dilution:	(49:49:2) 49 parts D7 Part 1 (A) + 49 parts D7 Part 2 (B) + 2 parts D7 Part 3
Diluent:	None
Test Temperature:	Room Temperature (Approximately 23°C ± 2°C)
Test Substance Application:	Pipette delivery
Test Substance Volume:	2.0 ml
Primary Neutralizer:	2% FBS EMEM supplemented with antibiotics
Secondary Neutralizer:	Sephacryl (S-1000 SF) gel filtration column
Carrier Dry Time:	44 minutes
Carrier Dry Temperature:	22.0°C
Carrier Dry Humidity:	41.4 – 42.8% relative humidity
Test Temperature:	22.0°C
Test Humidity:	42.8 – 43.4% relative humidity
Organic Soil Load:	5.0 ± 0.1% (v/v) Fetal Bovine Serum (FBS)
Neut. Control Hold Time:	18 minutes
Hold Time Temperature:	22.0°C
Hold Time Humidity:	43.1 – 43.3% relative humidity
Assay Conditions:	37 ± 2°C; 5 ± 1% CO_2
Assay Period:	6 days

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PROTOCOL CHANGES

Protocol Amendment(s)

None.

Protocol Deviation(s)

None.

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CONTROLS

Cytotoxicity Control

Sterile Glass Petri dish carriers (containing no virus films) were treated in the same manner as test carriers per test substance lot (2.0 ml of test substance delivered via pipette with total surface coverage), and held for the designated study contact time. Upon completion of the study contact time requested by the Study Sponsor, 2.0 ml of chemical neutralizer was applied to each carrier and the suspensions were promptly pipetted into pre-equilibrated Sephacryl (S-1000 SF) columns. The resulting filtrates were serially diluted (10-fold) as necessary to allow the determination of the extent of cytotoxicity in 0% serum EMEM or other appropriate media and applied in quadruplicate per dilution to the appropriate host cell culture monolayers prepared to suitable confluency in multi-well trays.

Test Substance Neutralization Control

A 0.900 ml aliquot of the Cytotoxicity Control filtrate (neutralized test substance) generated from the submitted test substance was used to determine the neutralization effectiveness of the prescribed neutralization method. A 0.900 ml aliquot of PBS was prepared as a control substance to determine if comparable levels of infectious viral units were recovered from the control and the neutralized test substance filtrate. Each Test System virus stock was diluted in order to add a low number (e.g. 1000 to 5000) of infective units of the respective test system into each neutralized test substance filtrate and PBS control substance preparation. The PBS control and neutralized test substance filtrate preparations were each inoculated with 0.100 ml of the low virus titer suspension and allowed to sit undisturbed for 10 to 20 minutes at room temperature. The two mixtures were then serially diluted (10-fold) in 0% serum EMEM or other appropriate media to determine the comparative levels of infectious viruses and applied in quadruplicate per dilution to the appropriate host cell culture monolayers prepared to suitable confluency in multi-well trays.

Cell Culture Control

To ensure that the host cells were not contaminated with bacteria, fungi, or any cytopathogenic viruses other than those used in the test and to confirm the viability of the cells during the incubation period of the assay, at least four host cell monolayers were left untreated and examined first at the end of the incubation period. Any obvious contamination or degeneration in such monolayers would have invalidated the virucidal efficacy assay.

Viral Inoculum Titer Control

To confirm that the host cell-line monolayers were susceptible to the test virus and to confirm the titer of the viral inoculum, an aliquot of the virus inoculum employed in the test was serially diluted (10-fold) in 0% serum EMEM or other appropriate media and applied in quadruplicate per dilution to the appropriate host cell culture monolayers prepared to suitable confluency in multi-well trays.

STUDY ACCEPTANCE CRITERIA

The U.S. EPA requires that the following measures are met to ensure the acceptability of virucidal efficacy data:

- A minimum of 4.00 $\log_{10}$ infective units/control carrier is recovered from each plate recovery control film.
- The cell culture control be absent of any obvious contamination or degradation of the monolayers.
- The virus titer control demonstrate obvious and or typical cytopathic effects on the monolayers.
- Comparable levels of infective units must be recovered from the neutralized test substance and neutralization verification control (1000-5000 infective units).
- Quantification of the plate recovery control, the virus recovery following test substance exposure, cytotoxicity control, test substance neutralization control, cell culture control, and virus stock titer controls are conducted at a minimum of four determinations per dilution for each assay system.
- Viral cytopathic effects (CPE) are to be distinguishable from cytotoxic effects related to or caused by test substance exposure.

The U.S. EPA performance criteria for disinfection follows:

- In the absence of cytotoxicity, if the product demonstrates complete inactivation of the virus at all dilutions, then efficacy is demonstrated by the test substance under the conditions evaluated.
- If cytotoxicity is observed, and if a $\geq$3.00-$\log_{10}$ reduction in viral titer is confirmed past the level of cytotoxicity, then efficacy is demonstrated by the test substance under the conditions evaluated.

Retesting Guidance:

- When a test passes and the $TCID_{50}$ of the plate recovery control is above 4.0, no retesting is necessary.
- When a test fails and $TCID_{50}$ of the plate recovery control is below 4.0, no retesting is necessary.
- When cytotoxicity is present and a $\geq$3.00 log reduction is observed, no retesting is necessary.
- When cytotoxicity is present and a $\leq$3.00 log reduction is observed, retesting may be necessary.

CALCULATIONS AND STATISTICAL ANALYSIS

The Spearman-Karber Method was used to calculate the Plate Recovery Control titer ($TCID_{50}$), the viral titer following test substance exposure ($TCLD_{50}$), and the titer of host cell cultures exhibiting cytotoxicity following test substance exposure ($TCCD_{50}$).

Determination of Viral and Cytotoxicity Titers

Viral Titer per ml = [X – d/2 + (d • S)], where:
X = $\log_{10}$ of dilution demonstrating complete infectivity or the lowest dilution performed
d = $\log_{10}$ of the dilution factor
S = sum of proportions of wells positive for CPE in all dilutions tested

Viral Titer per Carrier = Viral Titer per ml + Factor of Harvest Volume
where
Factor of Harvest Volume = Log_{10}[(Recovery Media Volume + Test Substance Volume)/1.0 ml]

Calculation of Virus Inactivation Due to Test Substance Exposure

Plate Recovery Control Log_{10} $TCID_{50}$ – Virus-Test Substance Film Log_{10} $TCLD_{50}$ = Log_{10} Reduction of Virus Due to Inactivation by Test Substance

STUDY RECORD AND TEST SUBSTANCE RETENTION

Data and Sample Retention

The study report and corresponding raw data will be held in the archives of Microchem Laboratory for at least 2 years after the study completion date. Afterward, Microchem reserves the right to transfer the documents to the Sponsor at Sponsor's expense. After record retention periods as described by section 195 of EPA and FDA GLP regulations have elapsed, unnecessary documentation may be destroyed.

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

RESULTS

Key: + = Virus recovered; O = Virus not recovered and/or no cytotoxicity observed; T = Toxicity observed

Table 1. The following were virucidal efficacy results for D7 Part 1 (A) + D7 Part 2 (B) + D7 Part 3 (D7 Part 1 (A) Lot: 17-390, D7 Part 2 (B) Lot: 17-393, and D7 Part 3 Lot: 20335) diluted at a ratio of (49:49:2) following a contact time of 9 minutes & 50 seconds ± 5 seconds against Feline calicivirus, Strain F-9, ATCC VR-782 supplemented to contain 5.0 ± 0.1% (v/v) Fetal Bovine Serum (FBS), dried on an inanimate surface. Testing conducted on 29DEC2017.

Dilution	Plate Recovery Control Replicate 1				Plate Recovery Control Replicate 2				Virus Test Film Lot: 17-390 + 17-393 Replicate 1				Virus Test Film Lot: 17-390 + 17-393 Replicate 2			
10^{-1}									T	T	T	T	T	T	T	T
10^{-2}	+	+	+	+	+	+	+	+	O	O	O	O	O	O	O	O
10^{-3}	+	+	+	+	+	+	+	+	O	O	O	O	O	O	O	O
10^{-4}	+	+	+	+	+	+	+	+	O	O	O	O	O	O	O	O
10^{-5}	+	O	O	+	O	O	O	O	O	O	O	O	O	O	O	O
10^{-6}	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
Per 1.00 ml	5.00 $\log_{10}$ $TCID_{50}$				4.50 $\log_{10}$ $TCID_{50}$				≤1.50 $\log_{10}$ $TCLD_{50}$				≤1.50 $\log_{10}$ $TCLD_{50}$			
Per Carrier	5.60 $\log_{10}$ $TCID_{50}$				5.10 $\log_{10}$ $TCID_{50}$				≤2.10 $\log_{10}$ $TCLD_{50}$				≤2.10 $\log_{10}$ $TCLD_{50}$			
Average Per Carrier	5.35 $\log_{10}$ $TCID_{50}$								≤2.10 $\log_{10}$ $TCLD_{50}$							
Average Log Reduction Per Carrier	≥3.25 log reduction															

RESULTS (cont.)

Key: + = Virus recovered; O = Virus not recovered and/or no cytotoxicity observed; T = Toxicity observed

Table 2. The following were virucidal efficacy results for D7 Part 1 (A) + D7 Part 2 (B) + D7 Part 3 (D7 Part 1 (A) Lot: 17-391, D7 Part 2 (B) Lot: 17-394, and D7 Part 3 Lot: 20335) diluted at a ratio of (49:49:2) following a contact time of 9 minutes & 50 seconds ± 5 seconds against Feline calicivirus, Strain F-9, ATCC VR-782 supplemented to contain 5.0 ± 0.1% (v/v) Fetal Bovine Serum (FBS), dried on an inanimate surface. Testing conducted on 29DEC2017.

Dilution	Plate Recovery Control Replicate 1				Plate Recovery Control Replicate 2				Virus Test Film Lot: 17-391 + 17-394 Replicate 1				Virus Test Film Lot: 17-391 + 17-394 Replicate 2			
10^{-1}									T	T	T	T	T	T	T	T
10^{-2}	+	+	+	+	+	+	+	+	O	O	O	O	O	O	O	O
10^{-3}	+	+	+	+	+	+	+	+	O	O	O	O	O	O	O	O
10^{-4}	+	+	+	+	+	+	+	+	O	O	O	O	O	O	O	O
10^{-5}	+	O	O	+	O	O	O	O	O	O	O	O	O	O	O	O
10^{-6}	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
Per 1.00 ml	5.00 $\log_{10}$ $TCID_{50}$				4.50 $\log_{10}$ $TCID_{50}$				≤1.50 $\log_{10}$ $TCLD_{50}$				≤1.50 $\log_{10}$ $TCLD_{50}$			
Per Carrier	5.60 $\log_{10}$ $TCID_{50}$				5.10 $\log_{10}$ $TCID_{50}$				≤2.10 $\log_{10}$ $TCLD_{50}$				≤2.10 $\log_{10}$ $TCLD_{50}$			
Average Per Carrier	5.35 $\log_{10}$ $TCID_{50}$								≤2.10 $\log_{10}$ $TCLD_{50}$							
Average Log Reduction Per Carrier	≥3.25 log reduction															

RESULTS (cont.)

Key: + = Virus recovered; O = Virus not recovered and/or no cytotoxicity observed; T = Toxicity observed

Table 3. The following was the viral stock inoculum titer determination for Feline calicivirus, Strain F-9, ATCC VR-782 supplemented to contain 5.0 ± 0.1% (v/v) Fetal Bovine Serum (FBS). Testing conducted on 29DEC2017.

Dilution	Viral Stock Inoculum Titer Determination			
10^{-3}	+	+	+	+
10^{-4}	+	+	+	+
10^{-5}	+	+	+	+
10^{-6}	O	+	O	O
10^{-7}	O	O	O	O
Per 1.00 ml	5.75 $\log_{10}$ $TCID_{50}$			

Table 4. The following were cytotoxicity control results to evaluate the effect of D7 Part 1 (A) + D7 Part 2 (B) + D7 Part 3 (D7 Part 1 (A) Lot: 17-390, D7 Part 2 (B) Lot: 17-393, and D7 Part 3 Lot: 20335) diluted at a ratio of (49:49:2) on CRFK host cell monolayers. Testing conducted on 29DEC2017.

Dilution	Cytotoxicity Control Lot: 17-390 +17-393			
10^{-1}	T	T	T	T
10^{-2}	O	O	O	O
10^{-3}	O	O	O	O
Per 1.00 ml	1.50 $\log_{10}$ $TCCD_{50}$			
Per Carrier	2.10 $\log_{10}$ $TCCD_{50}$			

RESULTS (cont.)

Key: + = Virus recovered; O = Virus not recovered and/or no cytotoxicity observed; T = Toxicity observed

Table 5. The following were cytotoxicity control results to evaluate the effect of D7 Part 1 (A) + D7 Part 2 (B) + D7 Part 3 (D7 Part 1 (A) Lot: 17-391, D7 Part 2 (B) Lot: 17-394, and D7 Part 3 Lot: 20335) diluted at a ratio of (49:49:2) on CRFK host cell monolayers. Testing conducted on 29DEC2017.

Dilution	Cytotoxicity Control Lot: 17-391 +17-394			
10^{-1}	T	T	T	T
10^{-2}	O	O	O	O
10^{-3}	O	O	O	O
Per 1.00 ml	1.50 $\log_{10}$ $TCCD_{50}$			
Per Carrier	2.10 $\log_{10}$ $TCCD_{50}$			

Table 6. The following were neutralization results of D7 Part 1 (A) + D7 Part 2 (B) + D7 Part 3 (D7 Part 1 (A) Lot: 17-390, D7 Part 2 (B) Lot: 17-393, and D7 Part 3 Lot: 20335) diluted at a ratio of (49:49:2) with Feline calicivirus, Strain F-9, ATCC VR-782 supplemented to contain 5.0 ± 0.1% (v/v) Fetal Bovine Serum (FBS). Testing conducted on 29DEC2017.

Dilution	Neutralization Inoculum Titer				PBS Neutralization Effectiveness Control				Test Neutralization Effectiveness Control Lot: 17-390 + 17-393			
10^{-1}					+	+	+	+	T	T	T	T
10^{-2}	+	+	+	+	+	+	+	+	+	+	+	O
10^{-3}	+	+	+	O	+	O	O	O	O	O	O	O
10^{-4}	O	O	O	O								
Per 1.00 ml	3.25 $\log_{10}$ $TCID_{50}$				2.75 $\log_{10}$ $TCID_{50}$				2.25 $\log_{10}$ $TCID_{50}$			

RESULTS (cont.)

Key: + = Virus recovered; O = Virus not recovered and/or no cytotoxicity observed; T = Toxicity observed

Table 7. The following were neutralization results of D7 Part 1 (A) + D7 Part 2 (B) + D7 Part 3 (D7 Part 1 (A) Lot: 17-391, D7 Part 2 (B) Lot: 17-394, and D7 Part 3 Lot: 20335) diluted at a ratio of (49:49:2) with Feline calicivirus, Strain F-9, ATCC VR-782 supplemented to contain 5.0 ± 0.1% (v/v) Fetal Bovine Serum (FBS). Testing conducted on 29DEC2017.

Dilution	Neutralization Inoculum Titer				PBS Neutralization Effectiveness Control				Test Neutralization Effectiveness Control Lot: 17-391 + 17-394			
10^{-1}					+	+	+	+	T	T	T	T
10^{-2}	+	+	+	+	+	+	+	+	+	+	+	+
10^{-3}	+	+	+	O	+	O	O	O	O	O	O	O
10^{-4}	O	O	O	O								
Per 1.00 ml	3.25 $\log_{10}$ $TCID_{50}$				2.75 $\log_{10}$ $TCID_{50}$				2.50 $\log_{10}$ $TCID_{50}$			

Table 8. CRFK host monolayer cell culture control for Feline calicivirus, Strain F-9, ATCC VR-782 supplemented to contain 5.0 ± 0.1% (v/v) Fetal Bovine Serum (FBS). Testing conducted on 29DEC2017.

Set	Results			
1	O	O	O	O
2	O	O	O	O
3	O	O	O	O

STUDY CONCLUSION

The purpose of the study was to determine the virucidal efficacy of D7 Part 1 (A) + D7 Part 2 (B) + D7 Part 3 (D7 Part 1 (A) Lots: 17-390 & 17-391, D7 Part 2 (B) Lots: 17-393 & 17-394, and D7 Part 3 Lot: 20335) diluted at a ratio of (49:49:2) against Feline calicivirus, Strain F-9, ATCC VR-782 supplemented to contain 5.0 ± 0.1% (v/v) Fetal Bovine Serum (FBS) at a contact time of 9 minutes & 50 seconds ± 5 seconds at ambient room temperature.

The average dried virus plate recovery titer obtained for Feline calicivirus, Strain F-9, ATCC VR-782 supplemented to contain 5.0 ± 0.1% (v/v) Fetal Bovine Serum (FBS) was 5.35 $\log_{10}$ $TCID_{50}$ per carrier. The prepared Feline calicivirus, Strain F-9, ATCC VR-782 treated virus films were reduced to ≤2.10 $\log_{10}$ $TCLD_{50}$ per carrier for D7 Part 1 (A) + D7 Part 2 (B) + D7 Part 3 (D7 Part 1 (A) Lots: 17-390 & 17-391, D7 Part 2 (B) Lots: 17-393 & 17-394, and D7 Part 3 Lot: 20335) diluted at a ratio of (49:49:2). The reduction in titer of Feline calicivirus, Strain F-9, ATCC VR-782 following the 9 minute & 50 second exposure for both tested lots was ≥3.25 $\log_{10}$.

The carriers designated as "Plate Recovery Control" during the course of testing yielded a viral titer of ≥4.00 $\log_{10}$ $TCID_{50}$ infectious viruses per carrier, thereby satisfying U.S. EPA study acceptance criteria (minimum of 4.00 $\log_{10}$ $TCID_{50}$ infectious viruses recovered).

Cytotoxic effects from the evaluated test substance D7 Part 1 (A) + D7 Part 2 (B) + D7 Part 3 (D7 Part 1 (A) Lots: 17-390 & 17-391, D7 Part 2 (B) Lots: 17-393 & 17-394, and D7 Part 3 Lot: 20335) diluted at a ratio of (49:49:2) were observed for the host cell monolayers (CRFK) to be 2.10 $\log_{10}$ $TCCD_{50}$ per carrier and ≥3.00-$\log_{10}$ reduction in viral titer was confirmed past the level of cytotoxicity.

Neutralization effectiveness control wells demonstrated confirmation of the chosen neutralization method for both evaluated lots of test substance D7 Part 1 (A) + D7 Part 2 (B) + D7 Part 3 (D7 Part 1 (A) Lots: 17-390 & 17-391, D7 Part 2 (B) Lots: 17-393 & 17-394, and D7 Part 3 Lot: 20335) diluted at a ratio of (49:49:2). No microbial contamination of any host cell cultures was observed during the course of the study.

The evaluated test substance, D7 Part 1 (A) + D7 Part 2 (B) + D7 Part 3 (D7 Part 1 (A) Lots: 17-390 & 17-391, D7 Part 2 (B) Lots: 17-393 &17-394, and D7 Part 3 Lot: 20335) diluted at a ratio of (49:49:2), demonstrated a ≥3.00 log reduction of Feline calicivirus, Strain F-9, ATCC VR-782 past the level of cytotoxicity thereby satisfying the U.S. EPA Product Performance Guidelines for Disinfectants for Use on Hard Surfaces outlined in OCSPP 810.2200.

This study was carried out in compliance with the approved Protocol Number P2046. All experimental controls met the established acceptance criteria unless otherwise noted on page 11.

STUDY CONCLUSION (cont.)

D7 Part 1 (A) + D7 Part 2 (B) + D7 Part 3 (D7 Part 1 (A) Lots: 17-390 & 17-391, D7 Part 2 (B) Lots: 17-393 & 17-394, and D7 Part 3 Lot: 20335) diluted at a ratio of (49:49:2) met the U.S. EPA Product Performance Guidelines for Disinfectants for Use on Hard Surfaces outlined in OCSPP 810.2200 and the success criteria detailed in the approved protocol when tested against Feline calicivirus, Strain F-9, ATCC VR-782 supplemented to contain 5.0 ± 0.1% (v/v) Fetal Bovine Serum (FBS) at a contact time of 9 minutes & 50 seconds ± 5 seconds.

REFERENCES

- *Annual Book of ASTM Standards*, Standard Test Method to Assess Virucidal Activity of Chemicals Intended for Disinfection of Inanimate, Nonporous Environmental Surfaces, Designation E1053. American Society for Testing Materials, 100 Barr Harbor Drive, West Conshohocken, PA 19428. 2011 Edition.
- *Annual Book of ASTM Standards*, Standard Practice for Use of Gel Filtration Columns for Cytotoxicity Reduction and Neutralization, Designation E1482. American Society for Testing Materials, 100 Barr Harbor Drive, West Conshohocken, PA 19428. 2012 Edition.
- U.S. EPA Product Performance Test Guidelines OCSPP 810.2200: Disinfectants for Use on Hard Surfaces – Efficacy Data Recommendations.
- Initial Virucidal Effectivenes Test Using Feline Calicivirus as Surrogate for Norovirus. U.S. EPA Antimicrobials Division, 1200 Pennsylvania Avenue, NW Washington DC 20460.

PROTOCOL

Protocol for GLP ASTM E1053 Method – P2046

Test Microorganism
Feline calicivirus, Strain F-9, ATCC VR-782
(U.S. EPA-Approved Human Norovirus Surrogate)

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

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

Test Substance: D7 Part 3
Lot Numbers: 20335

Data Requirement
U.S. EPA 40 CFR § 158
U.S. EPA OCSPP 810.2200

Study Sponsor
Brian Narducci
Decon 7
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Performing Laboratory
Microchem Laboratory
1304 W. Industrial Blvd.
Round Rock, Texas 78681

Protocol Number
P2046

Prepared By:
Kelli Jo Vagias, B.S.

Date
20DEC2017

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PROTOCOL (cont.)

I. Introduction

This document details the materials and procedure for evaluating the virucidal efficacy of a liquid disinfectant using the ASTM E1053 Method in accordance with Good Laboratory Practice Standards (GLPS) stipulated by U.S. EPA 40 CFR Part 160 and the U.S. EPA Product Performance Test Guidlines outlined in OCSPP 810.2200. This document also explains the terms and conditions of testing.

II. Purpose

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

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

The United States Environmental Protection Agency (U.S. EPA) requires that specific antimicrobial claims made for disinfectants for use on hard surfaces sold in the United States be supported by relevant test systems (microorganisms) outlined in the EPA Product Performance Test Guidelines, OCSPP 810.2200, Disinfectants for Use on Hard Surfaces Efficacy Data Recommendations and other EPA related guidance.

IV. Terms and Conditions

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

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

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

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

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

PROTOCOL (cont.)

V. Test Substance Identification, Characterization, and Handling

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

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

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

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

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

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

Special Handling Requirements — **None**

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

Test substances are handled as follows:
- The test substance is shaken or otherwise mixed well immediately prior to use (if applicable).
- The test substance is handled safely in accordance with the chemical risks it may pose, stated in the MSDS or by the Study Sponsor during the course of pre-study communication.

PROTOCOL (cont.)

VI. Study Parameters, Incorporated by References

Number of Tests Comprising the Study – **4 (2 lots of test substance, 2 replicates tested per lot)**
Carrier Type – **100 x 15 mm glass Petri dish**
Test Substance Form – **Dilution Required**
(49:49:2). 49 parts D7 Part 1 (A) + 49 parts D7 Part 2 (B) + 2 parts D7 Part 3
Test Substance Diluent — **None**
Test Temperature — **Room Temperature (Approximately 23°C ± 2 °C)**
Contact Time — **9 minutes & 50 seconds ± 5 seconds**
Organic Soil Load — **5 ± 0.1% (v/v) Fetal Bovine Serum (FBS)**
Volume of Test Substance Applied per Carrier – **2.0 ml**
Test Cell Culture/Assay Medium – **2% FBS EMEM supplemented with antibiotics**
Primary Neutralizer – **To be noted in final report**
Secondary Neutralizer – **Sephacryl (S-1000 SF) gel filtration columns**
Test Assay Incubation Period – **5-7 Days**
Test Assay Incubation Conditions – **37 ± 2°C, 5.0 ± 1% CO_2**

Proposed Experimental Start Date: **04DEC2017**
Proposed Experimental Termination Date: **11DEC2017**

VII. Test System (Microorganism)

Feline calicivirus, Strain F-9, ATCC VR-782 (U.S. EPA-Approved Human Norovirus Surrogate)

PROTOCOL (cont.)

VIII. Materials

- Pure stocks of test system (microorganism).
- Crandell-Rees Feline Kidney (CRFK) cells, ATCC CCL-94 (host cell system for Feline calicivirus) prepared to suitable confluence in an appropriate number of multi-well cell culture trays.
- Sufficient volume of test cell culture/assay medium for Feline calicivirus, Strain F-9, ATCC VR-782: 2% FBS EMEM plus antibiotics [100 µg/ml kanamycin sulfate solution and antibiotic-antimycotic solution (100 units/ml penicillin G, 100 µg/ml streptomycin, and 0.25 µg/ml amphotericin B)].
- Sufficient volume of 10% FBS EMEM plus antibiotics [100 µg/ml kanamycin sulfate solution and antibiotic-antimycotic solution (100 units/ml penicillin G, 100 µg/ml streptomycin, and 0.25 µg/ml amphotericin B)].
- Sufficient volume of 0% serum EMEM plus antibiotics [100 µg/ml kanamycin sulfate solution and antibiotic-antimycotic solution (100 units/ml penicillin G, 100 µg/ml streptomycin, and 0.25 µg/ml amphotericin B)].
- Sufficient volume of Phosphate Buffered Saline (PBS).
- Sufficient volume of DI water.
- Sufficient quantity of sterile glass Petri dish carriers (100 x 15mm).
- Sufficient quantity of pre-equilibrated Sephacryl (S-1000 SF) columns for test substance neutralization.
- Sufficient volume of the appropriate primary chemical neutralizer.
- Automatic pipettor (Pipet-Aid or similar) and various sizes of sterile serological pipets.
- Calibrated micropipette(s) and sterile micropipette tips containing aerosol barriers of suitable volumetric capacities.
- Sufficient quantity of sterile disposable cell scrapers.
- Incubator capable of maintaining the temperature range (37 ± 2°C) and atmospheric conditions (5 ±1% CO_2) appropriate for host cell assay incubation.
- Inverted light microscope.
- Certified digital timer.
- Certified satellite clock.
- Calibrated thermometer.
- Calibrated hygrometer.
- Autoclave.
- Vortex mixer.
- Stir plate(s).
- Stir bar(s).
- Scale with the ability to accurately weigh chemicals, compounds, and media used in the study.
- Sterile beaker(s) or other appropriate glassware for preparation of test substance.
- Appropriate volume of 95% ethanol.
- Appropriate volume of 500 ppm bleach.
- Centrifuge

PROTOCOL (cont.)

IX. Procedure

Preparation of Stock Virus

- The Feline calicivirus strain (F-9) to be used in the study is obtained from the American Type Culture Collection (ATCC) located in Manassas, Virginia.
- The virus strain(s) to be used in this study are readied by subjecting multiple cell culture flasks displaying cytopathic effect on ≥ 90% of the permissive host cell monolayers to at least three freeze thaw cycles to release virus from infected cells.
- After subjection to several freeze-thaw cycles, the contents of the cell culture flasks are combined and centrifuged at 1200 G for 10 minutes in order to remove cell debris.
- After centrifugation, the pellet is discarded and the supernatant is filtered using a 0.22μ filter.
- One milliliter aliquots are stored at approximately -70°C until the day of use, at which time the appropriate number of stock aliquots are removed, thawed, and used promptly in the assay. Viral stocks may be diluted as needed to reach an acceptable viral inoculum titer for study purposes.
- Prior to carrier inoculation, the test suspension is vortex mixed and diluted in sterile PBS (or another appropriate diluent), if applicable, to achieve $>1.0 \times 10^4$ infective units/carrier from dried, inoculated plate recovery control carriers.
- The stock virus may be titered on the appropriate host cell line, prior to efficacy testing, to determine stock virus concentration.

Preparation of Host Cell-Line Trays

- Once the host cell-line reaches a desired confluence, the cell-line is subcultured.
- Media is discarded from the culture flask(s) via serological pipette and the monolayers are rinsed with appropriate media (e.g. EMEM or DMEM) containing 0% serum to remove any residual serum and help facilitate trypsinization.
- 0.25% Trypsin-EDTA is added to the culture flask(s) and incubated under appropriate conditions until the monolayers begin to detach.
- Upon trypsinization of the monolayer(s), the appropriate complete cell culture growth medium is added to the flask(s) (e.g. 10% EMEM or 10% DMEM) to inactivate the trypsin.
- The contents of the flask(s) are transferred to a sterile conical and vortex mixed to obtain a homogenous suspension.
- A cell count is then performed using a hemocytometer and calculated.
- Host cells are added to the appropriate growth media and seeded at a concentration that will allow for 1.0×10^6 cells/tray.
- Trays are incubated no more than 48 hours at 37 ± 2°C, 5.0 ± 1% CO_2 to reach the desired confluence required for testing.

PROTOCOL (cont.)

Preparation of Test Carriers

- Clean glass Petri dish carriers (100 x 15 mm) are visually screened prior to use in testing, and flawed carriers are discarded.
- Before the test, carriers are soaked in 95% ethanol followed by multiple reverse osmosis water rinses.
- Carriers are autoclave sterilized and allowed to cool to room temperature prior to use.

Preparation of Chemical Neutralizer

- To ensure the cessation of antiviral activity at the conclusion of the selected contact time, a chemical neutralizer is applied to the appropriate carriers as required.
- The chemical neutralizer may be prepared by first creating a concentrated stock solution (e.g. a 3.0 ± 0.5% sodium thiosulfate solution prepared in DI/RO water).
- On the day of testing the concentrated stock solution may be diluted in 2% FBS EMEM supplemented with antibiotics in such a way as to create the final required concentration of chemical neutralizer. A sufficient volume will be prepared to allow for neutralization of test carriers and control carriers as appropriate.

Preparation of Sephacryl (S-1000 SF) Gel Filtration Columns

- Sephacryl (S-1000 SF) columns are constructed and equilibrated using three separate volumes of phosphate-buffered saline (PBS). Centrifugation of the columns for 3 to 4 minutes (600 x *g*) is performed to clear any residual liquid from the column prior to use.

Preparation of Test Substance

- Test substance is prepared by dilution.
  - The following ratio is used to prepare the test substance 49:49:2. This is equivalent to 49 parts D7 Part 1 (A) + 49 parts D7 Part 2 (B) + 2 parts D7 Part 3.
  - The solution is stirred or mixed well for 15-20 seconds.
- Other proportional volumes may be used as necessary for the conduct of the study.
- Test substance will be used within 3 hours of preparation.

PROTOCOL (cont.)

Preparation of Test Inoculum

- Prior to carrier inoculation, the test suspension is vortex mixed and diluted in sterile PBS (or another appropriate diluent), if applicable, to achieve >1.0 x 10^4 infective units/carrier from dried, inoculated carriers.
- The viral inoculum may then be supplemented with organic soil.
- Thawed, sterile fetal bovine serum is added to the viral test inoculum such that the final concentration is 5.0 ± 0.1% (v/v), if applicable.
- The viral inoculum-soil mixture is swirled gently to mix, if applicable.

Preparation of Virus Films

- The prepared inoculum virus stock suspension is vortexed thoroughly and 0.2 ml is placed on the inside bottom surface of each sterile glass Petri dish. A larger inoculum volume may be used as necessary in order to ensure an appropriate viral inoculum titer. The inoculum is then spread over the entire area of the carriers (10-in^2 equivalent surface) using a sterile cell scraper tool or bent pipette tip.
- The virus films are dried under ambient conditions in a laminar flow hood or other suitable chamber with the Petri dish covers removed, unless special drying conditions (e.g. specific temperature and/or humidity ranges) are required to lessen the levels of virus inactivation. The viral inoculum is allowed to dry for 20 minutes, or until the surface appears to be visibly dry. The temperature, humidity, and drying time period are recorded.
- Dried virus films are prepared and labeled according to the following designations:
  - Two Plate Recovery Control Carriers per test microorganism to determine the baseline dried virus titer.
  - Two Virus Test Carriers per test substance lot per test microorganism to determine the levels of infectious virus following exposure to the test substance at each contact time requested by the Study Sponsor.

Exposure of Virus Films to the Test Substance, and Processing of Treated Virus Films

- For each lot of the test substance, two dried film carrier is treated with 2.0 ml of the liquid product test substance. Carriers are then gently rotated to ensure complete coverage of the test substance over the entirety of each test surface.
- Carriers are held at ambient room temperature for the specified contact time. Upon completion of the study contact time requested by the Study Sponsor, 2.0 ml of chemical neutralizer is applied to each carrier and sterile cell scrapers are used to re-suspend the viral films. The suspensions are promptly pipetted into pre-equilibrated Sephacryl (S-1000 SF) columns.
- Syringe plungers may be used to release the initial volumes of filtrate, followed by centrifugation (3 to 4 minutes, 600 x *g*) to retrieve any residual liquid. Serial 10-fold dilutions are prepared using 0% serum EMEM or other appropriate media through to the appropriate dilution and applied in quadruplicate per dilution to the appropriate host cell culture monolayers prepared to suitable confluency in multi-well trays.

PROTOCOL (cont.)

Processing of the Plate Recovery Control Films

- Two plate Recovery Control film is processed per test microorganism to determine the baseline dried virus titer. The Plate Recovery Control film is generated as described above in "Preparation of Virus Films."
- After drying, 2.0 ml of 2% FBS EMEM, or other appropriate media, is overlayed on each control film.
- The carrier is then gently rotated to ensure complete coverage of the solution over the entirety of the surface. All other test conditions and parameters (e.g. exposure temperature) are the same as for the test virus films. The Plate Recovery Control is held for the same contact time used for the test carriers.
- Upon completion of the study contact time requested by the Study Sponsor, 2.0 ml of chemical neutralizer is applied to each carrier and sterile cell scrapers are used to re-suspend the viral films. The suspensions are promptly pipetted into pre-equilibrated Sephacryl (S-1000 SF) columns.
- Syringe plungers may be used to release the initial volumes of filtrate, followed by centrifugation (3 to 4 minutes, 600 x *g*) to retrieve any residual liquid. Serial 10-fold dilutions are prepared using 0% serum EMEM or other appropriate media through to the appropriate dilution and applied in quadruplicate per dilution to the appropriate host cell culture monolayers prepared to suitable confluency in multi-well trays.

Cytotoxicity Control

- Sterile Glass Petri dish carriers (containing no virus films) are treated in the same manner as test carriers per test substance lot (2.0 ml of test substance delivered via pipette with total surface coverage), and held for the designated study contact time.
- Upon completion of the study contact time requested by the Study Sponsor, 2.0 ml of chemical neutralizer is applied to each carrier and the suspensions are promptly pipetted into pre-equilibrated Sephacryl (S-1000 SF) columns.
- Syringe plungers may be used to release the initial volumes of filtrate, followed by centrifugation (3 to 4 minutes, 600 x *g*) to retrieve any residual liquid.
- The resulting filtrate is serially diluted (10-fold) as necessary to allow for the determination of the extent of cytotoxicity in 0% serum EMEM or other appropriate media and applied in quadruplicate per dilution to the appropriate host cell culture monolayers prepared to suitable confluency in multi-well trays.

Test Substance Neutralization Control

- A 0.900 ml aliquot of the Cytotoxicity Control filtrate (neutralized test substance) generated from the submitted test substance is used to determine the neutralization effectiveness of the prescribed neutralization method.
- A 0.900 ml aliquot of PBS is prepared as a control substance to determine if comparable levels of infectious viral units are recovered from the control and the neutralized test substance filtrate.
- Each Test System virus stock is diluted in order to add a low number (e.g. 1000 to 5000) of infective units of the respective test system into each neutralized test substance filtrate and PBS control substance preparation.
- The PBS control and neutralized test substance filtrate preparations are each inoculated with 0.100 ml of the low virus titer suspension and allowed to sit undisturbed for 10 to 20 minutes at room temperature.
- The two mixtures are then serially diluted (10-fold) in 0% serum EMEM or other appropriate media to determine the comparative levels of infectious viruses and applied in quadruplicate per dilution to the appropriate host cell culture monolayers prepared to suitable confluency in multi-well trays.

PROTOCOL (cont.)

Cell Culture Control

- To ensure that the host cells are not contaminated with bacteria, fungi, or any cytopathogenic viruses other than those used in the test and to confirm the viability of the cells during the incubation period of the assay, at least four host cell monolayers are left untreated, and examined at the end of the incubation period. Any obvious contamination or degeneration in such monolayers may invalidate the virucidal efficacy assay.

Virus Inoculum Titer Control

- To confirm that the host cell-line monolayers are susceptible to the test virus and to confirm the titer of the viral inoculum, an aliquot of the virus inoculum employeed in the test is serially diluted (10-fold) in 0% serum EMEM or other appropriate media and applied in quadruplicate per dilution to the appropriate host cell culture monolayers prepared to suitable confluency in multi-well trays.

Infectivity Assay Incubation

- All assay trays are incubated at 37 ± 2 °C (5 ± 1% CO_2) for a minimum of 30 minutes to facilitate virus-host cell adsorption. The trays may also be placed upon an orbital rotator during this incubation period, if feasible. Following incubation, each well receives ~1.0 ml of test/cell culture medium via pipette delivery. The cell culture assay trays are incubated at 37 ± 2 °C (5 ± 1% CO_2) for 5-7 days in a humidified CO_2 incubator.
- Assay trays may be examined regularly, with changes to healthy monolayers including viral cytopathic effects (CPE), cytotoxicity, and contamination clearly documented as such changes are observed.

PROTOCOL (cont.)

X. Calculations

- The Spearman-Karber Method is used to calculate the Plate Recovery Control titer ($TCID_{50}$), the viral titer following test substance exposure ($TCLD_{50}$), and the titer of host cell cultures exhibiting cytotoxicity following test substance exposure ($TCCD_{50}$).
- The $TCID_{50}$ (Tissue Culture Infectivity Dose) represents the endpoint dilution where 50% of the cell cultures exhibit cytopathic effects due to infection by the test virus. The dose required to kill 50% of the test viruses after the given exposure time is referred to as the Tissue Culture Lethal Dose ($TCLD_{50}$), and the endpoint dilution at which 50% of the host cell monolayers exhibit cytotoxicity is termed the Tissue Culture Cytotoxic Dose ($TCCD_{50}$). The $TCID_{50}$, $TCLD_{50}$, and $TCCD_{50}$ are determined according to the method of Spearman-Karber as follows:

  Viral Titer per 1.0 ml = $[X - d/2 + (d \bullet S)]$, where:
  X = $\log_{10}$ of dilution demonstrating complete infectivity or the lowest dilution performed
  d = $\log_{10}$ of the dilution factor
  S = sum of proportions of wells positive for CPE in all dilutions tested

  Viral Titer per Carrier, where:
  1. Factor of Harvest Volume = Log_{10}[(Recovery Media Volume + Test Substance Volume)/1.0 ml]
  2. Viral Titer per Carrier = Viral Titer per 1.0 ml Volume + Factor of Harvest Volume

  Calculation of Virus Inactivation Due to Test Substance Exposure

  Plate Recovery Control Log_{10} $TCID_{50}$ – Virus-Test Substance Film Log_{10} $TCLD_{50}$ = Log_{10} Reduction of Virus Due to Inactivation by Test Substance

PROTOCOL (cont.)

XI. Success Criteria

- The U.S. EPA requires that the following measures are met to ensure the acceptability of virucidal efficacy data:
  - A minimum of 4.00 $\log_{10}$ infective units/ control carrier is recovered from each plate recovery control film.
  - The cell culture control be absent of any obvious contamination or degradation of the monolayers.
  - The virus titer control demonstrate obvious and or typical cytopathic effects on the monolayers.
  - Comparable levels of infective units must be recovered from the neutralized test substance and neutralization verification control (1000-5000 infective units).
  - Quantification of the plate recovery control, the virus recovery following test substance exposure, cytotoxicity control, test substance neutralization control, cell culture control, and virus stock titer controls are conducted at a minimum of four determinations per dilution for each assay system.
  - Viral cytopathic effects (CPE) are to be distinguishable from cytotoxic effects related to or caused by test substance exposure.

- The U.S. EPA performance criteria for disinfection follows:
  - In the absence of cytotoxicity, if the product demonstrates complete inactivation of the virus at all dilutions, then efficacy is demonstrated by the test substance under the conditions evaluated.
  - If cytotoxicity is observed, and if a $\geq$3.00-$\log_{10}$ reduction in viral titer is confirmed past the level of cytotoxicity, then efficacy is demonstrated by the test substance under the conditions evaluated.

•Retesting Guidance
  - When a test passes and the $TCID_{50}$ of the plate recovery control is above 4.0, no retesting is necessary.
  - When a test fails and $TCID_{50}$ of the plate recovery control is below 4.0, no retesting is necessary.
  - When cytotoxicity is present and a $\geq$3.00 log reduction is observed, no retesting is necessary.
  - When cytotoxicity is present and a $\leq$3.00 log reduction is observed, retesting may be necessary.

XII. Reporting

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

XIII. Data and Sample Retention

- The study report and corresponding data sheets will be held in the archives of Microchem Laboratory for at least 2 years after the date of the final report and then may be destroyed. If the study is used by the Study Sponsor in support of a label claim, documentation may be returned to the Study Sponsor for archiving at Study Sponsor's expense.
- The test substance may be returned to the Study Sponsor at Study Sponsor's request and expense within 30 days of study completion. If the Study Sponsor does not request return of the sample, it will be destroyed >30 days after study completion.

PROTOCOL (cont.)

XIV. Quality Control

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

XV. References

- *Annual Book of ASTM Standards*, Standard Test Method to Assess Virucidal Activity of Chemicals Intended for Disinfection of Inanimate, Nonporous Environmental Surfaces, Designation E1053. American Society for Testing Materials, 100 Barr Harbor Drive, West Conshohocken, PA 19428. 2011 Edition.
- *Annual Book of ASTM Standards*, Standard Practice for Use of Gel Filtration Columns for Cytotoxicity Reduction and Neutralization, Designation E1482. American Society for Testing Materials, 100 Barr Harbor Drive, West Conshohocken, PA 19428. 2012 Edition.
- U.S. EPA Product Performance Test Guidelines OCSPP 810.2200: Disinfectants for Use on Hard Surfaces – Efficacy Data Recommendations.
- Initial Virucidal Effectiveness Test Using Feline Calicivirus as Surrogate for Norovirus. U.S. EPA Antimicrobials Devision, 1200 Pennsylvania Avenue, NW Washington DC 20460.

PROTOCOL (cont.)

Protocol for GLP ASTM E1053 Method – P2046

XVI. Protocol Approval

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

Study Sponsor/Representative Signature Approving Protocol

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

12/20/17
Date (DDMMMYYYY)

Kelli Jo Vagias, Study Director
Microchem Laboratory
1304 W. Industrial Blvd.
Round Rock, TX 78681

27DEC2017
Date (DDMMMYYYY)

Microchem Laboratory • 1304 W. Industrial Blvd. • Round Rock, Texas 78681 • (512) 310-8378

CERTIFICATE OF ANALYSIS

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

Certificate of Analysis D7 Part 1 8/7/2017

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

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

CERTIFICATE OF ANALYSIS (cont.)

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

8/7/2017

Certificate of Analysis D 7 Part 2

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

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