ACCURATUS
—LAB SERVICES—
THE ANTIMICROBIAL AUTHORITY

# FINAL STUDY REPORT

## STUDY TITLE

Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces

**Virus: Avian Influenza A (H7N9) virus**

## PRODUCT IDENTITY

D7 Part 1 (16-194) D7 Part 2 (040516-7) D7 Part 3 (18840)
D7 Part 1 (16-202) D7 Part 2 (040516-8) D7 Part 3 (18840)

## TEST GUIDELINE

OCSPP 810.2200

## PROTOCOL NUMBER

DSS01110416.AFLU.3

## AUTHOR

Mary J. Miller, M.T.
Study Director

## STUDY COMPLETION DATE

January 31, 2017

## 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

A22375



## 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: _____

_____
Title

_____ Date: _____
Signature

## 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 exception:

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

Submitter:_____ Date:_____

Sponsor:_____ Date:_____

Study Director: Mary J. Miller Date: 1-31-17
Mary J. Miller, M.T.

## QUALITY ASSURANCE UNIT SUMMARY

Study: Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces

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: Preparation of Virus Films | January 12, 2017 | January 12, 2017 | January 13, 2017 |
| Final Report | January 29, 2017 | January 30, 2017 | January 31, 2017 |

Quality Assurance Specialist: _____ Date: 1-31-17

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## STUDY PERSONNEL

STUDY DIRECTOR: Mary J. Miller, M.T.

Professional Personnel Involved:

| | |
|---|---|
| Shanen Conway, B.S. | - Manager, Virology Laboratory Operations |
| Erica Flinn, B.A. | - Virologist |

# STUDY REPORT

## GENERAL STUDY INFORMATION

**Study Title:** Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces

**Project Number:** A22375

**Protocol Number:** DSS01110416.AFLU.3

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

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

## TEST SUBSTANCE IDENTITY

**Test Substance Name:** D7 Part 1 (16-194) D7 Part 2 (040516-7) D7 Part 3 (18840)
D7 Part 1 (16-202) D7 Part 2 (040516-8) D7 Part 3 (18840)

**Lot/Batch(s):**

**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 I-III.

## STUDY DATES

**Date Sample Received:** December 1, 2016
**Study Initiation Date:** December 20, 2016
**Experimental Start Date:** January 12, 2017 (Start time: 3:34 p.m.)
**Experimental End Date:** January 19, 2017 (End time: 4:00 p.m.)
**Study Completion Date:** January 31, 2017

## OBJECTIVE

The objective of this study was to evaluate the virucidal efficacy of a test substance for registration of a product as a virucide. The test procedure was to simulate the way in which the product is intended to be used. This method is in compliance with the requirements of and may be submitted to the U.S. Environmental Protection Agency (EPA) and Health Canada.

## SUMMARY OF RESULTS

| | |
|---|---|
| Test Substance: | D7 Part 1 (16-194) D7 Part 2 (040516-7) D7 Part 3 (18840)<br>D7 Part 1 (16-202) D7 Part 2 (040516-8) D7 Part 3 (18840) |
| Dilution: | 1 (Part 1):1 (Part 2) + 2% of the total volume of Part 3<br>98.0 mL of Part 1 + 98.0 mL of Part 2 + 4.0 mL of Part 3<br>Applied as a trigger spray |
| Virus: | Avian Influenza A (H7N9) virus, Strain wildtype A/Anhui/1/2013<br>CDC # 2013759189 |
| Exposure Time: | 10 minutes |
| Exposure Temperature: | Room temperature (20.0°C) |
| Organic Soil Load: | 1% fetal bovine serum |
| Efficacy Result: | Two batches of D7 [D7 Part 1 (16-194) D7 Part 2 (040516-7) D7 Part 3 (18840) and D7 Part 1 (16-202) D7 Part 2 (040516-8) D7 Part 3 (18840)] met the performance requirements specified in the study protocol. The results indicate **complete inactivation** of Avian Influenza A (H7N9) virus under these test conditions as required by the U.S. EPA and Health Canada. |

## TEST SYSTEM

1. Virus

The wildtype A/Anhui/1/2013 strain of Avian Influenza A (H7N9) virus used for this study was obtained from the Centers for Disease Control and Prevention (CDC), Atlanta, GA (CDC # 2013759189). The stock virus was prepared by collecting the supernatant culture fluid from 75-100% infected culture cells. The cells were disrupted and cell debris removed by centrifugation at approximately 2000 RPM for ten minutes at approximately 4°C. The supernatant was removed, aliquoted, and the high titer stock virus was stored at ≤-70°C until the day of use. On the day of use, an aliquot of stock virus (Accuratus Lab Services Lot H7N9-4) was removed, thawed and maintained at a refrigerated temperature until used in the assay. The stock virus culture was adjusted to contain 1% fetal bovine serum as the organic soil load. The stock virus tested demonstrated cytopathic effects (CPE) typical of Influenza virus on MDCK cells.

2. Indicator Cell Cultures

Cultures of MDCK (canine kidney) cells were originally obtained from the American Type Culture Collection, Manassas, VA (ATCC CCL-34). The cells were propagated by Accuratus Lab Services personnel. The cells were seeded into multiwell cell culture plates and maintained at 36-38°C in a humidified atmosphere of 5-7% $CO_2$. On the day of testing, the cells were observed as having proper cell integrity and confluency, and therefore, were acceptable for use in this study.

All cell culture documentation was retained for the cell cultures used in the assay with respect to source, passage number, growth characteristics, seeding densities and the general condition of the cells.

3. Test Medium

The test medium used in this study was Minimum Essential Medium (MEM) supplemented with 2 µg/mL TPCK-trypsin, 25 mM HEPES, 0.2% bovine serum albumin (BSA) fraction V, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B.

## TEST METHOD

1. Preparation of Test Substance

Two batches of D7 were diluted 1 (Part 1):1 (Part 2) + 2% of the total volume of Part 3 and applied as a trigger spray, as requested by the Sponsor. Each batch of test substance was prepared in an individual Accuratus Lab Services trigger spray bottle.

One batch was prepared using 98.0 mL of D7 Part 1 (16-194) + 98.0 mL of D7 Part 2 (040516-7) + 4.0 mL of D7 Part 3 (18840) and the other batch was prepared using 98.0 mL of D7 Part 1 (16-202) + 98.0 mL of D7 Part 2 (040516-8) + 4.0 mL of D7 Part 3 (18840).

The prepared test substance was allowed to stand at least 5 minutes prior to testing and was used within 3 hours of preparation. Each batch of test substance was in solution as determined by visual observation and was at the exposure temperature prior to use.

2. Preparation of Virus Films

Films of virus were prepared by spreading 200 µL of virus inoculum uniformly over the bottoms of three separate 100 x 15 mm sterile glass petri dishes (without touching the sides of the petri dish). The virus films were dried at 20.0°C in a relative humidity of 11.4% until visibly dry (20 minutes).

3. Preparation of Sephadex Gel Filtration Columns

To reduce the cytotoxic level of the virus-test substance mixture prior to assay of virus, and/or to reduce the virucidal level of the test substance, virus was separated from the test substance by filtration through Sephadex LH-20 gel. On the day of testing, Sephadex columns were prepared by centrifuging the prepared Sephadex gel in sterile syringes for three minutes to clear the void volume. The columns were then ready to be used in the assay.

4. Input Virus Control (TABLE 1)

On the day of testing, the stock virus utilized in the assay was titered by 10-fold serial dilution and assayed for infectivity to determine the starting titer of the virus. The results of this control are for informational purposes only.

5. Treatment of Virus Films with the Test Substance (TABLE 2)

For each batch of test substance, one dried virus film was individually exposed for 10 minutes at room temperature (20.0°C) to the amount of spray released under use conditions. The carriers were sprayed until thoroughly wet, using 4 sprays at a distance of 6 to 8 inches, and held covered for the exposure time. The virus films were completely covered with the test substance. Just prior to the end of the exposure time, the plates were individually scraped with a cell scraper to resuspend the contents and at the end of the exposure time the virus-test substance mixtures were immediately passed through individual Sephadex columns utilizing the syringe plungers in order to detoxify the mixtures. The filtrates ($10^{-1}$ dilution) were then titered by 10-fold serial dilution and assayed for infectivity and/or cytotoxicity.

6. Treatment of Dried Virus Control Film (TABLE 1)
One virus film was prepared as previously described (paragraph 2). The virus control film was exposed to 2.00 mL of test medium in lieu of the test substance and held covered for 10 minutes at room temperature (20.0°C). Just prior to the end of the exposure time, the virus control was scraped with a cell scraper and at the end of the exposure time the virus mixture was immediately passed through a Sephadex column in the same manner as the test virus (paragraph 5). The filtrate ($10^{-1}$ dilution) was then titered by 10-fold serial dilution and assayed for infectivity.

7. Cytotoxicity Controls (TABLE 3)
Each batch of the test substance was sprayed as previously described onto separate sterile petri dishes and held covered for the 10 minute exposure time at room temperature (20.0°C). Just prior to the end of the exposure time, the plates were individually scraped with a cell scraper and at the end of the exposure time the contents were immediately passed through individual Sephadex columns utilizing the syringe plungers. The filtrates ($10^{-1}$ dilution) were then titered by 10-fold serial dilution and assayed for cytotoxicity. Cytotoxicity of the MDCK cell cultures was scored at the same time as the virus-test substance and virus control cultures.

8. Assay of Non-Virucidal Level of Test Substance (Neutralization Control) (TABLE 4)
Each dilution of the neutralized test substance (cytotoxicity control dilutions) was challenged with an aliquot of low titer stock virus to determine the dilution(s) of test substance at which virucidal activity, if any, was retained. Dilutions that showed virucidal activity were not considered in determining reduction of the virus by the test substance.

Using the cytotoxicity control dilutions prepared above, an additional set of indicator cell cultures was inoculated with a 100 µL aliquot of each dilution in quadruplicate. A 100 µL aliquot of low titer stock virus (approximately 3 infectious units) was inoculated into each cell culture well and the indicator cell cultures were incubated along with the test and virus control plates.

9. Infectivity Assays
The MDCK cell line, which exhibits cytopathic effect (CPE) in the presence of Avian Influenza A (H7N9) virus, was used as the indicator cell line in the infectivity assays. Cells in multiwell culture dishes were inoculated in quadruplicate with 100 µL of the dilutions prepared from test and control groups. The input virus control was inoculated in duplicate. Uninfected indicator cell cultures (cell controls) were inoculated with test medium alone. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% $CO_2$ in sterile disposable cell culture labware. The cultures were scored periodically for seven days for the absence or presence of CPE, cytotoxicity, and for viability.

10. Statistical Methods: Not applicable

## PROTOCOL CHANGES

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

**Protocol Deviations:**
No protocol deviations occurred during this study.

## DATA ANALYSIS

### Calculation of Titers

Viral and cytotoxicity titers are expressed as $-\log_{10}$ of the 50 percent titration endpoint for infectivity ($TCID_{50}$) or cytotoxicity ($TCD_{50}$), respectively, as calculated by the method of Spearman Karber.

$$\text{- Log of 1st dilution inoculated} - \left[\left(\left(\frac{\text{Sum of \% mortality at each dilution}}{100}\right) - 0.5\right) \times (\text{logarithm of dilution})\right]$$

### Calculation of Log Reduction

Dried Virus Control $Log_{10}$ $TCID_{50}$ – Test Substance $Log_{10}$ $TCID_{50}$ = Log Reduction

## STUDY ACCEPTANCE CRITERIA

### U.S. EPA and Health Canada Submission

A valid test requires 1) that at least 4 $log_{10}$ of infectivity be recovered from the dried virus control film; 2) that when cytotoxicity is evident, at least a 3-log reduction in titer is demonstrated beyond the cytotoxic level; 3) that the cell controls be negative for infectivity. **Note:** An efficacious product must demonstrate complete inactivation of the virus at all dilutions.

## RECORD RETENTION

### Study Specific Documents

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 the final study report.
7. Study-specific SOP deviations made during the study.

### Test Substance Retention

The test substance will be discarded following study completion per Sponsor approved protocol. It is the responsibility of the Sponsor to retain a sample of the test substance.

## REFERENCES

1. Annual Book of ASTM Standards, Section 11 Water and Environmental Technology Volume 11.05 Pesticides, Antimicrobials, and Alternative Control Agents; Environmental Assessment; Hazardous Substances and Oil Spill Response, E1053-11.
2. Annual Book of ASTM Standards, Section 11 Water and Environmental Technology Volume 11.05 Pesticides, Antimicrobials, and Alternative Control Agents; Environmental Assessment; Hazardous Substances and Oil Spill Response, E1482-12.
3. 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.
4. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, OCSPP 810.2200: Disinfectants for Use on Hard Surfaces - Efficacy Data Recommendations, September 4, 2012.
5. Diagnostic Procedures for Viral, Rickettsial, and Chlamydial Infections. Lennette, E.H., Lennette, D.A. and Lennette, E.T. editors. Seventh edition, 1995.
6. Blackwell, J.H., and J.H.S. Chen. 1970. Effects of various germicidal chemicals on HEP-2 cell culture and Herpes simplex virus. J. AOAC 53:1229-1236.
7. Health Canada, January 2014. Guidance Document – Disinfectant Drugs.
8. Health Canada, January 2014. Guidance Document – Safety and Efficacy Requirements for Hard Surface Disinfectant Drugs.

## STUDY RESULTS

Results of tests with two batches of D7 [D7 Part 1 (16-194) D7 Part 2 (040516-7) D7 Part 3 (18840) and D7 Part 1 (16-202) D7 Part 2 (040516-8) D7 Part 3 (18840)], diluted 1 (Part 1):1 (Part 2) + 2% of the total volume of Part 3 and applied as a trigger spray, exposed to Avian Influenza A (H7N9) virus in the presence of a 1% fetal bovine serum organic soil load at room temperature (20.0°C) for 10 minutes are shown in Tables 1-4. All cell controls were negative for test virus infectivity.

The titer of the input virus control was 6.00 $\log_{10}$. The titer of the dried virus control was 5.50 $\log_{10}$. Following exposure, test virus infectivity was not detected in the virus-test substance mixture for either batch at any dilution tested (≤2.50 $\log_{10}$). Test substance cytotoxicity was observed in both batches at 2.50 $\log_{10}$. The neutralization control (non-virucidal level of the test substance) indicates that the test substance was neutralized at ≤2.50 $\log_{10}$ for both batches. Taking the cytotoxicity and neutralization control results into consideration, the reduction in viral titer was ≥3.00 $\log_{10}$ for both batches.

## STUDY CONCLUSION

**Under the conditions of this investigation and in the presence of a 1% fetal bovine serum organic soil load, D7, diluted 1 (Part 1):1 (Part 2) + 2% of the total volume of Part 3 and applied as a trigger spray, demonstrated complete inactivation of Avian Influenza A (H7N9) virus following a 10 minute exposure time at room temperature (20.0°C) as required by the U.S. EPA and Health Canada.**

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: Virus Controls Results

### Input Virus Control Results and Results of Avian Influenza A (H7N9) Virus Dried on an Inanimate Surface Following a 10 Minute Exposure Time

| Dilution | Input Virus Control | Dried Virus Control |
|---|---|---|
| Cell Control | 0 0 | 0 0 0 0 |
| $10^{-1}$ | + + | + + + + |
| $10^{-2}$ | + + | + + + + |
| $10^{-3}$ | + + | + + + + |
| $10^{-4}$ | + + | + + + + |
| $10^{-5}$ | + + | + + + + |
| $10^{-6}$ | 0 + | 0 0 0 0 |
| $10^{-7}$ | 0 0 | 0 0 0 0 |
| $10^{-8}$ | 0 0 | 0 0 0 0 |
| $TCID_{50}$/100 µL | $10^{6.00}$ | $10^{5.50}$ |

(+) = Positive for the presence of test virus
(0) = No test virus recovered and/or no cytotoxicity present

## TABLE 2: Test Results

### Effects of D7 [D7 Part 1 (16-194) D7 Part 2 (040516-7) D7 Part 3 (18840) and D7 Part 1 (16-202) D7 Part 2 (040516-8) D7 Part 3 (18840)] Following a 10 Minute Exposure to Avian Influenza A (H7N9) Virus Dried on an Inanimate Surface

| Dilution | Avian Influenza A (H7N9) virus + D7 Part 1 (16-194) D7 Part 2 (040516-7) D7 Part 3 (18840) | Avian Influenza A (H7N9) virus + D7 Part 1 (16-202) D7 Part 2 (040516-8) D7 Part 3 (18840) |
|---|---|---|
| Cell Control | 0 0 0 0 | 0 0 0 0 |
| $10^{-1}$ | T T T T | T T T T |
| $10^{-2}$ | T T T T | T T T T |
| $10^{-3}$ | 0 0 0 0 | 0 0 0 0 |
| $10^{-4}$ | 0 0 0 0 | 0 0 0 0 |
| $10^{-5}$ | 0 0 0 0 | 0 0 0 0 |
| $10^{-6}$ | 0 0 0 0 | 0 0 0 0 |
| $10^{-7}$ | 0 0 0 0 | 0 0 0 0 |
| $10^{-8}$ | 0 0 0 0 | 0 0 0 0 |
| $TCID_{50}$/100 µL | $\leq 10^{2.50}$ | $\leq 10^{2.50}$ |

(0) = No test virus recovered and/or no cytotoxicity present
(T) = Cytotoxicity present

## TABLE 3: Cytotoxicity Control Results

### Cytotoxicity of D7 on MDCK Cell Cultures

| Dilution | Cytotoxicity Control D7 Part 1 (16-194) D7 Part 2 (040516-7) D7 Part 3 (18840) | Cytotoxicity Control D7 Part 1 (16-202) D7 Part 2 (040516-8) D7 Part 3 (18840) |
|---|---|---|
| Cell Control | 0 0 0 0 | 0 0 0 0 |
| $10^{-1}$ | T T T T | T T T T |
| $10^{-2}$ | T T T T | T T T T |
| $10^{-3}$ | 0 0 0 0 | 0 0 0 0 |
| $10^{-4}$ | 0 0 0 0 | 0 0 0 0 |
| $10^{-5}$ | 0 0 0 0 | 0 0 0 0 |
| $10^{-6}$ | 0 0 0 0 | 0 0 0 0 |
| $10^{-7}$ | 0 0 0 0 | 0 0 0 0 |
| $10^{-8}$ | 0 0 0 0 | 0 0 0 0 |
| $TCD_{50}$/100 µL | $10^{2.50}$ | $10^{2.50}$ |

(0) = No test virus recovered and/or no cytotoxicity present
(T) = Cytotoxicity present

## TABLE 4: Neutralization Control Results

### Non-Virucidal Level of the Test Substance (Neutralization Control)

| Dilution | Test Virus + Cytotoxicity Control D7 Part 1 (16-194) D7 Part 2 (040516-7) D7 Part 3 (18840) | Test Virus + Cytotoxicity Control D7 Part 1 (16-202) D7 Part 2 (040516-8) D7 Part 3 (18840) |
|---|---|---|
| Cell Control | 0 0 0 0 | 0 0 0 0 |
| $10^{-1}$ | T T T T | T T T T |
| $10^{-2}$ | T T T T | T T T T |
| $10^{-3}$ | + + + + | + + + + |
| $10^{-4}$ | + + + + | + + + + |
| $10^{-5}$ | + + + + | + + + + |
| $10^{-6}$ | + + + + | + + + + |
| $10^{-7}$ | + + + + | + + + + |
| $10^{-8}$ | + + + + | + + + + |

(+) = Positive for the presence of test virus after low titer stock virus added (neutralization control)
(0) = No test virus recovered and/or no cytotoxicity present
(T) = Cytotoxicity present

Results of the non-virucidal level control indicate that the test substance was neutralized at a $TCID_{50}$/100 µL of ≤2.50 $\log_{10}$ for both batches.

## ATTACHMENT I: Certificate of Analysis – D7 Part 1

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

November 28, 2016
Certificate of Analysis Decon 7 Part I

Decon7 Part 1 contains 2 quaternary ammonium compounds. The active [Alkyl Dimethylbenzeyl Ammonium Chloride] is present at 3.2% wt., N Alkyl pentamethy propane diamonium dichloride is present at 1% wt. Total Quat. concentration is assayed using method BCQCSP-2.11.

| batch number | % wt. Total quaternary ammonium compounds |
|---|---|
| 16-194 | 4.13 |
| 16-202 | 4.24 |
| 16-222 | 4.18 |
| | |
| | |
| | |
| | |
| | |
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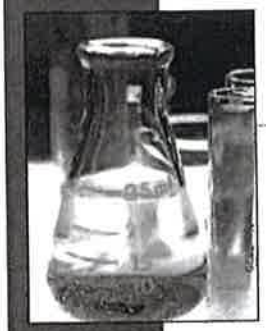
| | |
| | |

EXACT COPY
INITIALS DATE 1-31-17

## ATTACHMENT II: Certificate of Analysis – D7 Part 2



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

November 28, 2016

Certificate of Analysis Decon 7 Part 2

Part 2 is assayed for %wt.$H_2O_2$ using method BCQCSP – 6.44. Expiration date to all product is 11/28/2017.

| batch number | %wt. $H_2O_2$ | LCL | UCL |
|---|---|---|---|
| 040516-7 | 7.601 | 7.51 | 8.3 |
| 040516-8 | 7.556 | 7.51 | 8.3 |
| 040516-9 | 7.561 | 7.51 | 8.3 |

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**ATTACHMENT III: Certificate of Analysis – D7 Part 3**

## Allan Chemical Corporation

235 Margaret King Avenue
Ringwood, New Jersey 07456

Telephone: 1(973) 962-4014
Fax: 1(973) 962-6820
E-Mail: allanchem@allanchem.com

## CERTIFICATE OF ANALYSIS

**Product:** DIACETIN
**Manufacture Date:** 10/13/15
**Suggested Re-test Date:** 10/12/17
**Batch No:** 18840

| Property | Units | Specification | Results |
|---|---|---|---|
| Appearance | Clear liquid, free from suspended matter. | | Satisfactory |
| Colour | Hazen | 10 max | 3 |
| Acidity (as acetic acid) | % w/w | 0.05 max | 0.023 |
| Saponification Value | mg KOH/g | 542 – 605 | 574.2 |
| Water Content | % w/w | 0.2 max | 0.03 |
| Specific Gravity @ 20/20°C | | 1.180 – 1.195 | 1.186 |

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(For Laboratory Use Only)
Accuratus Lab Services Project # A22375
Test Substance Tracking # TS120116.DSS01
ISP 12-21-16
12-2-16

ACCURATUS LAB SERVICES

**PROTOCOL**

# Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces

Virus: Avian Influenza A (H7N9) virus

**PROTOCOL NUMBER**

DSS01110416.AFLU.3

**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**

November 4, 2016

EXACT COPY
INITIALS MN DATE 1-31-17





1285 Corporate Center Drive, Suite 110 • Eagan, MN 55121 • 877.287.8378 • 651.379.5510 • www.accuratuslabs.com

## Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces

**PURPOSE**

The purpose of this study is to evaluate the virucidal efficacy of a test substance for registration of a product as a virucide. The test procedure is to simulate the way in which the product is intended to be used. 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), Health Canada and Australian Therapeutic Goods Administration (TGA).

**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 November 22, 2016. Verbal results may be given upon completion of the study with a written report to follow on the proposed completion date of December 20, 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, because of 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 regulatory agency of its submission 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 virucidal claim for a disinfectant intended for use on hard surfaces be supported by appropriate scientific data demonstrating the efficacy of the test substance against the claimed virus. Each agency will accept adequate data generated by any appropriate technique in support of a virucidal efficacy claim. This is accomplished by treating the target virus with the disinfectant (test substance) under conditions, which simulate as closely as possible, in the laboratory, the actual conditions under which the disinfectant is designed to be used. For disinfectant products intended for use on hard surfaces (dry, inanimate environmental surfaces), a carrier method is used in the generation of the supporting virological data. The MDCK cell line, which supports the growth of the Avian Influenza A (H7N9) virus, will be used in this study. The experimental design in this protocol meets these requirements.

## TEST PRINCIPLE

A film of virus, dried on a glass surface, is exposed to the test substance for a specified exposure time. At the end of the exposure time, the virucidal and cytotoxic activities are removed from the virus-test substance mixture, and the mixture is assayed for viral infectivity by an accepted assay method. Appropriate virus, test substance cytotoxicity, and neutralization controls are run concurrently.

## STUDY DESIGN

Dried virus films will be prepared in parallel and used as follows:

The appropriate number of films for each batch of test substance assayed per exposure time requested.

The appropriate number of films for virus control titration (titer of virus after drying) per exposure time requested.

The inoculated carriers are exposed to the test substance for the Sponsor specified exposure time. At the end of the specified exposure time, resuspended virus-test substance mixtures will be detoxified and made non-virucidal by immediately adding the contents to a Sephadex gel filtration column followed by 10-fold serial dilutions in test medium. Each dilution is inoculated into indicator cell cultures. The resuspended virus control film and each batch of test substance alone will be treated in exactly the same manner. For analysis of infectivity, cultures will be held for the appropriate incubation period at the end of which time cultures will be scored for the presence of the test virus. Cultures will be monitored at that time for cell viability. Uninfected indicator cell cultures will be carried in parallel and similarly monitored. For analysis of cytotoxicity, the viability of cultures inoculated with dilutions of each test and cytotoxicity control will be determined. In addition to the above titrations for infectivity and cytotoxicity, the residual virucidal activity of the test substance after neutralization will be determined by adding a low titer of stock virus to each dilution of the test substance (cytotoxicity control dilutions). The resulting mixtures of dilutions are assayed for infectivity in order to determine the dilution(s) of test substance at which virucidal activity, if any, is retained.

## VIRUS

The wildtype A/Anhui/1/2013, CDC # 2013759189strain of Avian Influenza A (H7N9) virus to be used for this study was obtained from the Centers for Disease Control and Prevention (CDC, Atlanta, GA). Stock virus is prepared by collecting the supernatant culture fluid from 75-100% infected culture cells. The cells are disrupted and cell debris removed by centrifugation. The supernatant is removed, aliquoted, and the high titer stock virus may be stored at ≤ -70°C until the day of use. Alternate methods of viral propagation may be utilized based on the growth requirements of the virus. The propagation method will be specified in the raw data and in the report. On the day of use the appropriate number of aliquots are removed, thawed, combined (if applicable) and maintained at a refrigerated temperature until used in the assay. **Note:** If the Sponsor requests an organic soil load challenge, fetal bovine serum (FBS) or the requested organic soil will be incorporated into the stock virus aliquot. The stock virus aliquot will be adjusted to yield the percent organic soil load requested.

## INDICATOR CELL CULTURES

Cultures of MDCK (canine kidney) cells were originally obtained from the American Type Culture Collection, Manassas, VA (ATCC CCL-34). The cells are propagated by Accuratus Lab Services personnel. The cells are seeded into multiwell cell culture plates and maintained at 36-38°C in a humidified atmosphere of 5-7% $CO_2$. The confluency of the cells will be appropriate for the test virus. MDCK cells obtained from an alternate, reputable source may be used. The source of the cells will be specified in the final report.

All cell culture documentation is retained for the cell cultures used in this assay with respect to source, passage number, growth characteristics, seeding densities and the general condition of the cells.

## TEST MEDIUM

The test medium used for this assay is Minimum Essential Medium (MEM) supplemented with 0-10% (v/v) heat inactivated fetal bovine serum. The medium may also be supplemented with one or more of the following: 10 µg/mL gentamicin, 100 units/mL penicillin, 2.5 µg/mL amphotericin B, 1.0-2.0 mM L-glutamine, and 0.5 – 5 µg/mL trypsin. The composition of the test medium may be altered based on the virus and/or cells. The composition of the medium will be specified in the raw data and in the report.

## PREPARATION OF TEST SUBSTANCE

The dilution of test substance(s) will be used as recommended by the Sponsor. The product will be pre-equilibrated to the desired test temperature if applicable.

## PREPARATION OF VIRUS FILMS

Films of virus will be prepared by spreading 200 µL of virus inoculum uniformly over the bottom of the appropriate number of 100 X 15 mm sterile glass petri dishes (without touching the sides of the petri dish). The virus will be air-dried at 10°C-30°C until visibly dry (≥20 minutes). A calibrated timer will be used for timing the drying. The drying conditions (temperature and humidity) will be appropriate for the test virus for the purpose of obtaining maximum survival following drying. The actual drying conditions, drying time and calibrated timer used will be clearly documented.

One dried virus film per batch of test substance will be assayed unless otherwise requested.

## TEST METHOD

### Preparation of Sephadex Gel Filtration Columns

To reduce the cytotoxic level of the virus-test substance mixture prior to assay of virus, and/or to reduce the virucidal level of the test substance, virus is separated from the test substance by filtration through Sephadex gel. The type of Sephadex used will be specified in the final report. On the day of testing, Sephadex columns are prepared by centrifuging the prepared Sephadex gel in sterile syringes for three minutes to clear the void volume. The columns are now ready to be used in the assay.

### Input Virus Control

On the day of testing, the stock virus utilized in the assay will be titered by 10-fold serial dilution and assayed for infectivity to determine the starting titer of the virus. The results of this control are for informational purposes only.

### Treatment of Virus Films with the Test Substance

For each batch of test substance assayed, the appropriate number of dried virus films are individually exposed to a 2.0 mL aliquot of the use dilution of the test substance (liquid products), or to the amount of spray released under use conditions (spray products) and held covered for the specified exposure time(s) and temperature. A calibrated timer will be used for timing the exposure. The actual temperature will be recorded. Just prior to the end of the exposure time, the plates are individually scraped with a cell scraper to resuspend the contents and at the end of the exposure time the virus-test substance mixtures are immediately passed through individual Sephadex columns utilizing the syringe plunger in order to detoxify the mixture. The filtrate ($10^{-1}$ dilution) is then titered by serial dilution and assayed for infectivity and/or cytotoxicity. To further aid in the removing of the cytotoxic effects of the test substance to the indicator cell cultures, individual dilutions may be passed through additional individual Sephadex columns.

**Treatment of Dried Virus Control Film**
The appropriate number of virus films are prepared as described previously for each exposure time assayed. The virus control films are run in parallel to the test virus but a 2.0 mL aliquot of test medium is added in lieu of the test substance. The virus control films are held covered and exposed to the test medium for the same exposure time and at the same exposure temperature as the test films are exposed to the test substance. A calibrated timer will be used for timing the exposure and the actual temperature will be recorded. Just prior to the end of the exposure time, the virus films are individually scraped as previously described and at the end of the exposure time the mixtures are immediately passed through individual Sephadex columns utilizing the syringe plunger. The filtrate ($10^{-1}$ dilution) is then titered by serial dilution and assayed for infectivity. If additional Sephadex columns were used to further reduce the cytotoxic effects in the test substance assay, the same dilutions of the virus control will be passed through additional individual Sephadex columns.

**Cytotoxicity Control**
A 2.0 mL aliquot of each batch of test substance (liquid products) or the amount of the test substance recovered when sprayed onto a sterile petri dish (spray products), is filtered through a Sephadex column utilizing the syringe plunger and the filtrate is diluted serially in medium and inoculated into cell cultures for assay of cytotoxicity concurrently with the virus control and test substance-treated virus samples. For spray products, the cytotoxicity control will be held covered for the longest requested exposure time at the requested exposure temperature. A calibrated timer will be used for timing the exposure. If additional Sephadex columns were used to further reduce the cytotoxic effects in the test substance assay, the same dilutions of the cytotoxicity control will be passed through additional individual Sephadex columns.

**Assay of Non-Virucidal Level of Test Substance (Neutralization Control)**
Each dilution of the neutralized test substance (cytotoxicity control dilutions) will be challenged with an aliquot of low titer stock virus to determine the dilution(s) of test substance at which virucidal activity, if any, is retained. Dilutions that show virucidal activity will not be considered in determining reduction of the virus by the test substance.

Using the cytotoxicity control dilutions prepared above, an additional set of indicator cell cultures will be inoculated with a 100 µL aliquot of each dilution in quadruplicate. A 100 µL aliquot of low titer stock virus will be inoculated into each cell culture well and the indicator cell cultures will be incubated along with the test and virus control plates.

**Infectivity Assays**
The MDCK cell line, which exhibits cytopathic effect (CPE) in the presence of Avian Influenza A (H7N9) virus, will be used as the indicator cell line in the infectivity assays. Cells in multiwell culture dishes will be inoculated in quadruplicate with 100 µL of the dilutions prepared from test and control groups. The input virus control will be inoculated in duplicate. Uninfected indicator cell cultures (cell controls) will be inoculated with test medium alone. The cultures are incubated at 36-38°C in a humidified atmosphere of 5-7% $CO_2$ in sterile disposable cell culture labware for approximately seven days. Periodically throughout the incubation time the cultures will be microscopically observed for the absence or presence of CPE, cytotoxicity and for viability. The observations will be recorded on the raw data worksheets; only the results from the final observations will be reported.

## DATA ANALYSIS

**Calculation of Titers**
Viral and cytotoxicity titers will be expressed as $-\log_{10}$ of the 50 percent titration endpoint for infectivity ($TCID_{50}$) or cytotoxicity ($TCD_{50}$), respectively, as calculated by the method of Spearman Karber.

$$-\text{Log of 1st dilution inoculated} - \left[\left(\left(\frac{\text{Sum of \% mortality at each dilution}}{100}\right) - 0.5\right) \times (\text{logarithm of dilution})\right]$$

**Calculation of Log Reduction**
Dried Virus Control $Log_{10}$ $TCID_{50}$ – Test Substance $Log_{10}$ $TCID_{50}$ = Log Reduction

If multiple dried virus control replicates are performed, the average titer of the replicates will be calculated and the average titer will be used to calculate the log reduction in viral titer of the individual test replicates.

**PROCEDURE FOR IDENTIFICATION OF THE TEST SYSTEM**
The specialized virucidal testing section of Accuratus Lab Services maintains Standard Operating Procedures (SOPs) relative to virucidal efficacy testing studies. Virucidal 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 virus and cell stocks for purposes of identification, receipt and use of chemical reagents including cell culture medium components, etc. These procedures are designed to document each step of virucidal 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 virucidal efficacy test is assigned a unique Project Number when the Study Director initiates the protocol for the study. This number is used for identification of the test culture plates, etc. during the course of the test. Test culture plates are also labeled with reference to the test virus, experimental start date, and test product. These measures are designed to document the identity of the test system.

**METHOD FOR CONTROL OF BIAS:** N/A

**STUDY ACCEPTANCE CRITERIA**
Only the applicable acceptance criteria and references for the regulatory agency reviewing the data will be included in the final report.

**U.S. EPA, Health Canada, and Australian TGA Submission**
A valid test requires 1) that at least 4 $\log_{10}$ of infectivity be recovered from the dried virus control film; 2) that when cytotoxicity is evident, at least a 3-log reduction in titer is demonstrated beyond the cytotoxic level; 3) that the cell controls be negative for infectivity. If any of the previous requirements are not met, the test may be repeated under the current protocol number. **Note:** An efficacious product must demonstrate complete inactivation of the virus at all dilutions.

If the test substance fails to meet the test acceptance criteria and the dried virus control fails to meet the control acceptance criteria, the study is considered valid and no repeat testing is necessary, unless requested by the Sponsor.

If any portion of the protocol is executed incorrectly warranting repeat testing, the test may be repeated under the current protocol number.

**FINAL REPORT**
The report will include, but not be limited to, identification of the sample and date received, dates on which the test was initiated and completed, identification of the virus strain used and composition of the inoculum, description of cells, medium and reagents, description of the methods employed, tabulated results, calculated titers for infectivity and cytotoxicity, and a conclusion as it relates to the purpose of the test. A draft report may be requested by the Sponsor. The final report will be prepared once the Sponsor has reviewed the draft report and notified the Study Director to complete the study.

**PROTOCOL CHANGES**
If it becomes necessary to make changes in the approved protocol, the revision and reasons for change 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.

**TEST SUBSTANCE RETENTION**

Test substance retention shall be the responsibility of the Sponsor. Unused test substance will be **discarded** following study completion unless otherwise requested.

**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 the 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.

**PROPOSED STATISTICAL METHODS:** N/A

## REFERENCES

1. Annual Book of ASTM Standards, Section 11 Water and Environmental Technology Volume 11.05 Pesticides, Antimicrobials, and Alternative Control Agents; Environmental Assessment; Hazardous Substances and Oil Spill Response, E1053-11.
2. Annual Book of ASTM Standards, Section 11 Water and Environmental Technology Volume 11.05 Pesticides, Antimicrobials, and Alternative Control Agents; Environmental Assessment; Hazardous Substances and Oil Spill Response, E1482-12.
3. 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.
4. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, OCSPP 810.2200: Disinfectants for Use on Hard Surfaces – Efficacy Data Recommendations, September 4, 2012.
5. Diagnostic Procedures for Viral, Rickettsial, and Chlamydial Infections. Lennette, E.H., Lennette, D.A. and Lennette, E.T. editors. Seventh edition, 1995.
6. Blackwell, J.H., and J.H.S. Chen. 1970. Effects of various germicidal chemicals on HEP-2 cell culture and Herpes simplex virus. J. AOAC 53:1229-1236.
7. Health Canada, January, 2014. Guidance Document - Disinfectant Drugs.
8. Health Canada, January, 2014. Guidance Document - Safety and Efficacy Requirements for Hard Surface Disinfectant Drugs.
9. Australian Therapeutic Goods Administration (TGA), February 1998. Guidelines for the Evaluation of Sterilants and Disinfectants.
10. Australian Therapeutic Goods Administration (TGA), February 1998. Therapeutic Goods Order No. 54: Standard for Disinfectants and Sterilants.
11. Australian Therapeutic Goods Administration (TGA), March 1997. Therapeutic Goods Order No. 54A: Amendment to Standard for Disinfectants and Sterilants (TGO 54).
12. Australian Therapeutic Goods Administration (TGA), July 2005. Draft Guidelines for the Evaluation of Household/Commercial and Hospital Grade Disinfectants.

## STUDY INFORMATION

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

**Test Substance (Name and Batch Number - exactly as it should appear on final report):**
D7 PART 1 (16-194) D7 PART 2 (040516-8) D7 PART 3 (18810)
(16-202) (040516-4) (1)

***Testing at the lower certified limit (LCL) for the hardest-to-kill virus on your label is required for registration.***

**Product Description**
- ☒ Quaternary ammonia
- ☐ Iodophor
- ☐ Peracetic acid
- ☒ Peroxide
- ☐ Sodium hypochlorite
- ☐ Other_____

**Approximate Test Substance Active Concentration (upon submission to Accuratus Lab Services):**
D7 PART 1 (3.20%) D7 PART 2 (7.9%) D7 PART 3 (99%)
*(This value is used for neutralization planning only. This value is not intended to represent characterization values.)*

**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**
- (1) ☒ No dilution required, Use as received (RTU)
- ☐ *Dilution(s) to be tested:
  _____ defined as _____ + _____
  (example: 1 oz/gallon) (amount of test substance) (amount of diluent)
  - ☐ 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
  - (1) ☒ Other See attachment for product preparation.

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

**Test Virus:** Avian Influenza A (H7N9) virus

**Exposure Time:** 10 Minutes

**Exposure Temperature:** ☒ Room temperature (to be based on regulatory agency of submission)
☐ Other:_____°C (please specify range)

**Directions for application of aerosol/spray products:**
- (1) ☒ Spray instructions are not applicable.

Trigger spray application:
- ☒ Spray carriers using 3 sprays, or until thoroughly wet, at a distance of 6 to 8 inches.
- ☐ Spray carriers using _____ sprays at a distance of _____ to ____ inches/cm. (circle one)

Aerosol spray application:
- ☐ Spray carriers for _____ seconds, or until thoroughly wet, at a distance of _____ to ____ inches/cm.

**Organic Soil Load**
- ☒ 1% fetal bovine serum (minimum level that can be tested)
- ☐ 5% fetal bovine serum
- ☐ Other_____

(1) = corrected per 12-7-16 email. MM 12-20-16

**Number of Carriers to be Tested**
- ☑ One (typical for U.S. EPA submission)
- ☐ Five (required for broad-spectrum virucidal claims for Health Canada submission)

**SPRAY BOTTLES USED IN TESTING (section only applicable for spray products)**
*To ensure expected levels of product are delivered, it is recommended that the Sponsor provide the spray bottles used in testing. Please indicate the desired source of the sprayer bottles used in testing:*
- ☐ Sprayer(s) and bottle(s) are provided by the Sponsor
- ☑ General purpose spray bottle(s) are to be provided by Accuratus Lab Services
- ☑ The spray nozzle(s) are provided by the Sponsor and general purpose bottle(s) will be provided by Accuratus Lab Services

(2)

**REGULATORY AGENCY(S) THAT MAY REVIEW DATA**
- ☑ U.S. EPA
- ☑ Health Canada
- ☐ Therapeutic Goods Administration (Australian TGA)
- ☐ Not applicable - For internal/other use only (Efficacy result will be based on U.S. EPA requirements)

**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)

**PROTOCOL MODIFICATIONS**
- ☑ Approved without modification
- ☐ Approved with modification

**PROTOCOL ATTACHMENTS**
Supplemental Information Form Attached - ☑ Yes ☑ No

(2) (2)

**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: received 12-7-16 mm 12-20-16
- ☐ 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).

(2) = Corrected per 12-7-16 email - includes mm 12-20-16
using Accuratus Lab Services trigger-spray bottles and an attachment.
mm 12-20-16

**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

(3) ☑ **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
☒ 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
☐ 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.*

(3) = corrected per 12-7-16 email. MM 12-20-16

**APPROVAL SIGNATURES**

***SPONSOR:***

NAME: Mr. Joe Drake TITLE: President/Founder

SIGNATURE: _____ DATE: 11/28/16

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

Brian Narducci (BNarducci@decon7.com)

***Accuratus Lab Services:***

NAME: Mary J. Miller
Study Director

SIGNATURE: Mary J. Miller DATE: 12-20-16
Study Director

ACCURATUS
—LAB SERVICES—
THE ANTIMICROBIAL AUTHORITY

ACCURATUS
—LAB SERVICES—

**Attachment to protocol DSS01110416.AFLU.3** mm 12-20-16 **Page 1 of 1**

On the day of each test, the product will be prepared and used within 3 hours of preparation by the following instructions:

Prepare the product 1 (part 1):1 (part 2) + 2% of the total volume of part 3. Examples for testing:

-To prepare 500 mls of test substance, add 245 mls of part 1 + 245 mls of part 2 + 10 mls of part 3.

-To prepare 100 mls of test substance, add 49 mls of part 1 + 49 mls of part 2 + 2 mls of part 3.

Once all three parts have been combined, mix and allow to stand at least 5 minutes prior to testing. Alternate dilutions may be made as appropriate.