STUDY TITLE
GLP AOAC Use-Dilution Method Modified for Fungi

Study Identification Number
GLP1919

Protocol Number
P2108

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 number: 20335)

Test Microorganism
Aspergillus niger ATCC 6275

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

Author
Nicholas Garcia, B.S.
Study Director

Study Completion Date
06JUN2018

Testing Facility
Microchem Laboratory
1304 W. Industrial Blvd.
Round Rock, TX 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 is made for any information contained in this study on the basis of its falling within the scope of FIFRA sec. 10(d)(1)(A), (B), or (C).

Company: _____

Agent/Submitter: _____

Title: _____

Date: _____

Signature: _____

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study meets U.S. Environmental Protection Agency's Good Laboratory Practice Standards and requirements for 40 CFR § 160 with the following exception:

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

Study Director

Company: Microchem Laboratory

Name: Nicholas Garcia, B.S.

Title: Study Director

Signature: _____ Date: 06JUN2025

Study Sponsor

Company: Decon 7

Name: Brian Narducci

Title: Study Sponsor

Signature: _____ Date: _____

Submitter

Company:

Name:

Title:

Signature: _____ Date: _____

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QUALITY ASSURANCE STATEMENT

Study Title: GLP AOAC Use-Dilution Method Modified for Fungi

Study ID#: GLP1919

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	13APR2018	13APR2018	13APR2018
Draft Report	18MAY2018	25MAY2018	25MAY2018
Final Report	05JUN2018	05JUN2018	05JUN2018

Quality Assurance Unit:

Signature: Travis Chesser Date: 06JUN2018

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

PERSONNEL INVOLVED IN THE STUDY

Study Director
Name: Nicholas Garcia, B.S.
Company: Microchem Laboratory
Title: Study Director

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

Assisting Personnel
Name: Victoria Sanchez, B.S.
Company: Microchem Laboratory
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Name: Jorge Cavazos, B.S.
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Company: Microchem Laboratory
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FINAL STUDY REPORT SUMMARY

Study Title
GLP AOAC Use-Dilution Method Modified for Fungi

Study Identification Number
GLP1919

Protocol Number
P2108

Test Microorganism
Aspergillus niger ATCC 6275

Study Sponsor
Brian Narducci
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8541 East Anderson Drive, Suite 106
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Testing Facility
Microchem Laboratory
1304 W. Industrial Blvd.
Round Rock, Texas 78681

Study Director
Nicholas Garcia, B.S.

Study Completion Date
06JUN2018

Study Objective
To determine, using the AOAC Use-Dilution Method Modified for Fungi, the antimicrobial efficacy of D7 (Lots: 17-390, 17-393, 20335, and Lots: 17-391, 17-394, 20335) against *Aspergillus niger* ATCC 6275 at a contact time of 9 minutes 50 seconds ± 5 seconds and a contact temperature of 20°C ± 1°C in the presence of 5% Fetal Bovine Serum.

Study Conclusion in Brief
D7 (Lots: 17-390, 17-393, 20335 and Lots: 17-391, 17-394, 20335) met the EPA OCSPP 810.2200 success criteria for disinfection when tested against *Aspergillus niger* ATCC 6275 at a contact time of 9 minutes 50 seconds ± 5 seconds and a contact temperature of 20°C ± 1°C in the presence of 5% Fetal Bovine Serum.

FINAL STUDY REPORT

Important Dates

Study Initiation Date:	13APR2018
Experimental Start Date/Time:	13APR2018 / 1111
Experimental Termination Date/Time:	09MAY2018 / 0826

Test Substance Information

Name: D7 Part 1 (A)
Lots: 17-390

Active Ingredient (concentration):	Alkyl Dimethylbenzyl Ammonium Chloride 3.04%

17-391

Active Ingredient (concentration):	Alkyl Dimethylbenzyl Ammonium Chloride 3.06%
Date of Manufacture:	01AUG2017
Date Received:	05SEP2017
Expiration Date:	01AUG2018

Name: D7 Part 2 (B)
Lots: 17-393

Active Ingredient (concentration):	Hydrogen Peroxide 7.528%

17-394

Active Ingredient (concentration):	Hydrogen Peroxide 7.469%
Date of Manufacture:	28JUL2017
Date Received:	05SEP2017
Expiration Date:	28JUL2018

Name: D7 Part 3
Lot: 20335

Non-Active Ingredient :	Diacetain (Concentration not necessary)
Date of Manufacture:	14SEP2016
Date Received:	05SEP2017
Expiration Date:	14SEP2018

Form: Liquid, Dilution required. For both combinations of D7 products, (Lots: 17-390, 17-393, 20335 and Lots: 17-391, 17-394, 20335) a dilution was required to achieve a ratio of 49:49:2 and was prepared in the following manner: 73.5 parts of D7 Part 1 (A) (Lots:17-390, 17-391) to 73.5 parts of D7 Part 2 (B) (Lots:17-393, 17-394) to 3 parts of D7 Part 3 (Lot: 20335) for a total of 150 mL of diluted product per combination of lots.

Storage Conditions: Ambient Temperature under fluorescent lighting or in a cabinet.

FINAL STUDY REPORT (cont.)

Test Parameters

Microorganism:	*Aspergillus niger* ATCC 6275
Culture Manipulation:	Test culture was diluted 1:10 for D7 (Lots: 17-390, 17-393, 20335 and Lots: 17-391, 17-394, 20335) in sterile phosphate buffered saline supplemented to contain 0.1% Triton X-100. Test culture was used to inoculate carriers within 30 minutes of manipulation.
Number of Test Carriers:	10 carriers per combination of lots
Carrier Type:	Stainless steel penicylinder carriers free of visible damage
Test Substance Dilution:	Test substance was diluted at a ratio of 49:49:2 (73.5 parts D7 Part 1 (A) + 73.5 parts D7 Part 2 (B) + 3 parts D7 Part 3). The solution was mixed well for 15-20 seconds and used within 3 hours of preparation.
Contact Time:	9 minutes 50 seconds ± 5 seconds
Organic Soil Load:	5% Fetal Bovine Serum
Test Temperature:	20°C ± 1°C
Neutralization Broth:	Lots: 17-390, 17-393, 20335 • Letheen Broth supplemented with 0.1% Sodium Thiosulfate,1.0% Tween 80, and 1% Catalase Lots: 17-391, 17-394, 20335 • Letheen Broth supplemented with 0.25% Lecithin,1.0% Tween 80, and 1% Catalase
Carrier Soak Time:	15 minutes ± 2 minutes
Carrier Dry Time:	40 minutes ± 2 minutes or until visibly dry
Carrier Dry Temperature:	36°C ± 1°C
Incubation Temperature:	30°C ± 2°C
Incubation Time(s):	Lots: 17-390, 17-393, 20335 • Enumeration plates: 46 hours and 51 minutes • Tubes: 7 days Lots: 17-391, 17-394, 20335 • Enumeration plates: 50 hours and 18 minutes • Tubes: 7 days

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PROTOCOL CHANGES

Protocol Amendment(s)

The signed protocol (P2108) was amended to include the following calculations used in the study. At the time of study initiation, the protocol did not specify the calculations to be used when interpreting data.

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

$$\frac{(\text{Average CFU for } 10^{-3}) + (\text{Average CFU for } 10^{-4}) + (\text{Average CFU for } 10^{-5})}{10^{-3} + 10^{-4} + 10^{-5}} = \text{CFU/ml}$$

$$[(\text{CFU/ml}) \times 10\text{ml}] = \text{CFU/Carrier}$$

$$\text{Mean Log Density} = \frac{(\text{Log}_{10}\text{ CFU/Carrier Pooled Pre Carriers} + \text{Log}_{10}\text{ CFU/Carrier Pooled Post Carriers})}{2}$$

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

All remaining testing parameters are to be followed as stated in Protocol P2108.

Protocol Deviation(s)

A deviation from the approved protocol was noted on 16APR2018. The library stock cultures were prepared on Potato Dextrose Agar slants, a deviation from the protocol, which states library stock cultures are maintained on Sabouraud Dextrose Agar slants. This deviation was not thought to affect the outcome of the study because sufficient growth of the pure target microorganism was observed on the slants and carrier counts were within range of protocol specifics.

A deviation from the approved protocol (P2108) was noted on 20APR2018 with regards to the neutralization broth used for the study. The neutralizer was updated from Letheen Broth supplemented with 0.1% Sodium Thiosulfate, 1.0% Tween 80, and 1.0% Catalase to Letheen Broth supplemented with 0.25% Lecithin, 1.0% Tween 80, and 1.0% Catalase, a deviation from the protocol. The testing performed for D7 (Lots:17-391, 17-394, and 20335) on 13APR2018 yielded insufficient recovery from the neutralizer effectiveness test, indicative of incomplete neutralization of the active ingredient of the test substance at the specified contact time. This deviation was deemed necessary for the outcome of the study, the study was successfully repeated on 02MAY2018, as growth of target microorganism was observed in neutralization tube.

CONTROLS

Enumeration of Inoculated Test Carriers
Following the conclusion of the dry time, dried inoculated carriers were assayed in two sets of three; one set of three immediately prior to conducting the test, and one set immediately following the test. Each carrier was aseptically transferred individually to a sterile subculture/neutralization test tube. These test tubes were placed into a beaker, which was filled with water to approximately the level of liquid in the tubes, and held by hand in a sonicator so that the beaker bottom did not touch the bottom of the sonicator and all 3 liquid levels were approximately equal, and sonicated for 1 minute ± 5 seconds, timed with a certified digital timer. Within 2 hours of sonication, each set of three test tubes (pre-test and post-test) were pooled then enumerated using standard dilution and plating techniques.

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

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

Media Sterility Controls
A tube containing only subculture/neutralization broth was incubated alongside the test to confirm neutralization/subculture sterility.
A plate containing growth medium was incubated alongside the enumeration plates to confirm plating media sterility.
Plates containing 0.100 ml of phosphate buffered saline, culture dilution media, and organic soil load were incubated alongside test enumeration plates to confirm media sterility.

Neutralization Control
A sterile uninoculated carrier was treated with the test substance similar to the treatment performed during the test. After the specified contact time had elapsed, the carrier was transferred, to a sterile subculture/neutralization broth test tube. After transfer, the test tube was inoculated with approximately 10 – 100 CFU of test microorganism (obtained by serial dilution) and incubated along with the other test tubes. An additional subculture/neutralization broth tube was inoculated and incubated alongside the test as a positive comparison. The inoculum was plated in duplicate to verify the number of CFU added and incubated alongside the test enumeration plates.

Test Microorganism Purity Control
A loopful of the test microorganism used in this study was subcultured to an appropriate growth agar medium and incubated alongside enumeration plates to morphologically confirm the presence of a pure culture at the time of test.

STUDY ACCEPTANCE CRITERIA

The experimental success (controls) criteria follow:

- The test microorganism must demonstrate a mean log density of at least 4.0 corresponding to 1×10^4 CFU/Carrier and not above 5.0 corresponding to a mean log density of 1×10^5 CFU/Carrier.
- The media sterility control test tubes are negative for growth.
- Viability growth control test tubes are positive for growth.
- The carrier sterility control test tubes are negative for growth.
- The neutralization control subculture/neutralization tubes are positive for growth.
- The neutralization test subculture/neutralization tubes are positive for growth.
- The neutralization control inoculum demonstrates 10-100 CFU.
- The test microorganism purity streak shows pure culture for growth.
- Additional media sterility controls are negative for growth.

The EPA performance criterion for disinfection follows:

- If 1 or more non-control subculture/neutralization test tubes are confirmed positive for *Aspergillus niger* ATCC 6275 growth after incubation, then efficacy is not demonstrated by the test substance under the conditions evaluated.

Retesting guidance for disinfection follows:

- When a test passes and the $\log_{10}$ density of the test carriers is above 5.0, no retesting is necessary.
- When a test fails and the $\log_{10}$ density of the test carriers is below 4.0, no retesting is necessary.
- When a test passes and the $\log_{10}$ density of the test carriers is below 4.0, retesting is necessary.
- When a test fails and the $\log_{10}$ density of the test carriers is above 5.0, retesting may be conducted.

CALCULATIONS AND STATISTICAL ANALYSIS

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

$$\frac{(\text{Average CFU for } 10^{-3}) + (\text{Average CFU for } 10^{-4}) + (\text{Average CFU for } 10^{-5})}{10^{-3} + 10^{-4} + 10^{-5}} = \text{CFU/ml}$$

$$[(\text{CFU/ml}) \times 10\text{ml}] = \text{CFU/Carrier}$$

$$\text{Mean Log}_{10}\text{ Density} = \frac{(\text{Log}_{10}\text{ CFU/carrier Pooled Pre Carriers}) + (\text{Log}_{10}\text{ CFU/carrier Pooled Post Carriers})}{2}$$

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

STUDY RECORD AND TEST SUBSTANCE RETENTION

Study Record Retention

The study report and corresponding data sheets will be held in the archives of Microchem Laboratory for at least 2 years after the date of the final report. Afterward the two-year period, Microchem Laboratory will contact the Study Sponsor for further archive instructions. If the study is used by the Study Sponsor in support of label claim, documentation may be returned to the Study Sponsor for archiving at Study Sponsor's expense.

Test Substance Retention

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

RESULTS

Table 1

The following were the carrier enumeration results for D7 (Lots: 17-390, 17-393, 20335). Testing conducted on 13APR2018.

Test Microorganism	Test Substance	Carriers	CFU/Carrier	Log_{10} Density	Mean Log_{10} Density
A. niger ATCC 6275	D7 (Lots: 17-390, 17-393, 20335)	Pre Treatment	4.82E+04	4.68	4.71
		Post Treatment	5.50E+04	4.74	

Table 2

The following were the carrier enumeration results for D7 (Lots: 17-391, 17-394, 20335). Testing conducted on 02MAY2018.

Test Microorganism	Test Substance	Carriers	CFU/Carrier	Log_{10} Density	Mean Log_{10} Density
A. niger ATCC 6275	D7 (Lots: 17-391, 17-394, 20335)	Pre Treatment	4.00E+04	4.60	4.62
		Post Treatment	4.23E+04	4.63	

Table 3

The following were efficacy test results for D7 (Lots: 17-390, 17-393, 20335) when tested against *A. niger* ATCC 6275 at a contact time of 9 minutes 50 seconds ± 5 seconds in the presence of 5% Fetal Bovine Serum. Testing conducted on 13APR2018.

Test Microorganism	Test Substance	Number of Carriers Tested	Number of Positive Subculture/Neutralizer Test Tubes
A. niger ATCC 6275	D7 (Lots: 17-390, 17-393, 20335)	10	0

RESULTS (cont.)

Table 4

The following were efficacy test results for D7 (Lots: 17-391, 17-394, 20335) when tested against *A. niger* ATCC 6275 at a contact time of 9 minutes 50 seconds ± 5 seconds in the presence of 5% Fetal Bovine Serum. Testing conducted on 02MAY2018.

Test Microorganism	Test Substance	Number of Carriers Tested	Number of Positive Subculture/Neutralizer Test Tubes
A. niger ATCC 6275	D7 (Lots: 17-391, 17-394, 20335)	10	0

Table 5

The following were the neutralization results for D7 (Lots: 17-390, 17-393, 20335). Neutralization results were in compliance with the aforementioned study acceptance criteria. The parallel subculture/neutralization broth control tube demonstrated positive growth indicative of the target microorganism. Testing conducted on 13APR2018.

Test Microorganism	Test Substance	Plate Counts (CFU)	Average Inoculum Concentration	Neutralization Verification Result
A. niger ATCC 6275	D7 (Lots: 17-390, 17-393, 20335)	18 / 21	19.5	Positive Growth

Table 6

The following were the neutralization results for D7 (Lots: 17-391, 17-394, 20335). Neutralization results were in compliance with the aforementioned study acceptance criteria. The parallel subculture/neutralization broth control tube demonstrated positive growth indicative of the target microorganism. Testing conducted on 02MAY2018.

Test Microorganism	Test Substance	Plate Counts (CFU)	Average Inoculum Concentration	Neutralization Verification Result
A. niger ATCC 6275	D7 (Lots: 17-391, 17-394, 20335)	55 / 52	53.5	Positive Growth

RESULTS (cont.)

Table 7
The following were the incubation conditions for the D7 (Lots: 17-390, 17-393, 20335) test materials incubated in this study. Testing conducted on 13APR2018.

Incubation Temperature Range	Test Materials	Incubation Duration
30°C ± 2°C	Media Sterility Controls, Carrier Enumeration Plates, and Purity Streak Plate	46 Hours 51 Minute
	Test Carrier Subculture/Neutralizer Tubes, Neutralization Verification Tubes, Carrier Sterility, Viability Control, Media Sterility Tube	7 Days
	Presumptive Positive Confirmation Plates	N/A

Table 8
The following were the incubation conditions for the D7 (Lots: 17-391, 17-394, 20335) test materials incubated in this study. Testing conducted on 02MAY2018.

Incubation Temperature Range	Test Materials	Incubation Duration
30°C ± 2°C	Media Sterility Controls, Carrier Enumeration Plates, and Purity Streak Plate	50 Hours 18 Minute
	Test Carrier Subculture/Neutralizer Tubes, Neutralization Verification Tubes, Carrier Sterility, Viability Control, Media Sterility Tube	7 Days
	Presumptive Positive Confirmation Plates	N/A

RESULTS (cont.)

Table 9

The following were the incubation conditions for the test microorganism used in this study. Testing conducted on 13APR2018 and 02MAY2018.

Test Culture	Transfer Date	Incubation Temperature Ranges	Culture Incubation Time
Initial Culture From Microbial Library Stock (Transfer 1)	22MAR2018	30 °C ± 2 °C	7 Days
Daily Transfer to SDA Plates (Transfer 2)	30MAR2018		10 Days

Table 10

The following were the results for sterility, growth, and purity controls conducted during the study.

Study Controls	Result
Carrier Sterility Control Tube	No Growth Observed
Viability Control Tube	Growth-Target Microorganism
Neutralization Media Control Tube	No Growth Observed
Growth Media Control Plate	No Growth Observed
Dilution Media Sterility Plate	No Growth Observed
Culture Dilution Media Sterility Plate	No Growth Observed
Organic Soil Load	No Growth Observed
Microorganism Purity Plate	Pure-Target Microorganism

STUDY CONCLUSION

For Study Identification Number GLP1919, test substances D7 (Lots: 17-390, 17-393, 20335 and Lots: 17-391, 17-394, 20335) were tested against *Aspergillus niger* ATCC 6275. A total of 10 contaminated carriers were exposed to each combination of lots of D7 test substance for a contact time of 9 minutes 50 seconds ± 5 seconds at a test temperature of 20°C ± 1°C in the presence of 5% Fetal Bovine Serum and then chemically neutralized.

The study was carried out in compliance with the approved protocol (P2108), and all experimental controls met the established acceptance criteria, unless noted otherwise on page 10 of this report.

D7 (Lots: 17-390, 17-393, 20335 and Lots: 17-391, 17-394, 20335) disinfected 10 out of 10 carriers within 9 minutes 50 seconds ± 5 seconds.

D7 (Lots: 17-390, 17-393, 20335 and Lots: 17-391, 17-394, 20335) 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.

REFERENCES

- "Association of Official Analytical Chemists, International." AOAC Official Method 955.17. Fungicidal Activity of Disinfectants.
- US EPA Product Performance Test Guidelines OCSPP 810.2200: Disinfectants for Use on hard Surfaces-- Efficacy Data Recommendations

PROTOCOL

Protocol for GLP AOAC Use Dilution Method Modified for Fungi
Protocol - P2108

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Test Microorganism(s)
Aspergillus niger ATCC 6275

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 Part 158
U.S. EPA OCSPP 810.2200

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Protocol Number
P2108

Author
Nicholas Garcia, B.S.

Date
16MAR2018

PROTOCOL (cont.)

I. Introduction

This document details the materials and procedure for evaluating the efficacy of a water soluble disinfectant using the AOAC Use-Dilution Method in accordance with Good Laboratory Practice Standards (GLPS) stipulated by 40 CFR 160. This document also explains the terms and conditions of testing.

II. Purpose

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

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

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

IV. Terms and Conditions

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

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

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

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

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

PROTOCOL (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.

PROTOCOL (cont.)

Test substances and devices are handled as follows:

- The test substance is stored at ambient (room) temperature under fluorescent lighting or in a cabinet.
- The test substance must be shaken vigorously or otherwise mixed well immediately prior to use.
- 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.

VI. Study Parameters, Incorporated by References

Number of Tests Comprising the Study — **2 (1 Test per Test Substance Lot)**
Carrier Type — **Stainless Steel Penicylinder**
Number of Carriers per Test Substance — **10 Carriers per Lot of Test Substance**
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 — **20 ± 1°C**
Contact Time — **9 minutes 50 seconds ± 5 seconds**
Organic Soil Load — **5 ± 0.1% (v/v) Fetal Bovine Serum (FBS)**
Neutralization Broth — **Letheen Broth Supplemented with 0.1% Sodium Thiosulfate,1.0% Tween 80, and 1% Catalase**

Proposed Experimental Start Date: **19MAR2018**
Proposed Experimental Termination Date: **19APR2018**

VII. Test System (Microorganism)

Aspergillus niger ATCC 6275

VIII. Materials

- Pure culture of the test system (microorganism).
- Sufficient quantity sterile 8 ± 1 mm od, 6 ± 1 mm id, 10 ± 1 mm length, type 304 stainless steel penicylinders free of visible flaws. For a 10 carrier test, at least 19 carriers are necessary per microorganism and lot of product tested (10 test carriers, 6 inoculum control carriers, 1 neutralization control carrier, and 1-2 viability control carriers). Extra carriers may be prepared for use in the study.
- Sufficient volume of reagent grade 1N NaOH solution.
- Sufficient quantity of clean, sterile 100×15 mm sterile Petri dishes.
- Sufficient quantity of sterile 9 cm Whatman #2 (or equivalent) filter paper rounds.
- Sufficient clean, sterile 25×100 mm test tubes.
- Sufficient volume of sterile phosphate-buffered saline or 0.85% saline
- Sufficient number of sterile Petri dishes containing approximately 15 ml solidified, sterile Sabouraud Dextrose Agar for enumeration of diluted microbial suspensions. Alternatively molten Sabouraud Dextrose Agarr tempered to approximately 45°C may be used for pour plating.
- Sufficient volume of sterile fetal bovine serum.
- Sufficient number of 25×150 mm test tubes containing 10 ml sterile subculture neutralization broth.

PROTOCOL (cont.)

- Two or more bent wire transfer hooks of a type that can be flame-sterilized quickly yet are strong enough to fully support the weight of a penicylinder during transfer from one tube to the next.
- Bunsen burner, microbiological incinerator, or micro-torch as appropriate to ensure rapid and complete flame-sterilization of transfer hooks.
- Sufficient quantity of micropipettes and appropriately sized sterile micropipette tips.
- Automatic pipettor (Pipette-Aid or similar) and various sizes of sterile serological pipettes.
- Thermometer (for submersion in an equilibrated test tube to indicate the temperature of the test substance during the test).
- Incubators capable of sustaining temperatures of 36°C ± 1°C and 30°C ± 2°C.
- Forceps.
- Appropriate volume of 95% ethanol.
- Wire inoculating loop.
- Sufficient number of test tube racks.
- Sonicator.
- Certified satellite clock.
- Certified digital timer.
- Water Bath capable of maintaining the appropriate test temperature.

IX. Procedure

Preparation of Test Tubes and Test Substance

- All test tubes that will receive test substance are thoroughly cleaned and steam sterilized prior to use.
- 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.
- 10 ml of the prepared test substance is transferred by sterile disposable serological pipet, or other means as appropriate, into each 25 x 100 mm test tube designated for that purpose, and equilibrated to test temperature for ≥10 minutes prior to initiating testing or recording test substance temperature.
- This substance is to be used within 3 hours of preparation.

Preparation of Test Tubes for Subculture/Neutralization and Incubation of Treated Carriers

- Before the test begins, the subculture/neutralization test tubes are prepared by cleaning, followed by the addition of 10 ml of an appropriate subculture neutralizing medium and steam sterilized prior to use.

Preparation of Test Carriers

- Before the test, clean stainless steel carriers are soaked in fresh 1N NaOH for at least 12 hours.
- Carriers are thoroughly rinsed using multiple tap-water rinses followed by a double R/O water rinse.
- An aliquot of rinse water from the final R/O water rinse is collected, and mixed with 2—3 drops phenolphthalein. If alkalinity is observed (rinse water turns pink) the carriers are re-rinsed until alkalinity is no longer observed.
- Carriers are distributed into an appropriate autoclavable container, covered with deionized or reverse osmosis water and steam sterilized.
- Carriers are allowed to cool to room temperature prior to use in the study.

PROTOCOL (cont.)

- Prior to use in the study, carriers are observed for flaws and flawed carriers are discarded.

Preparation of Test Culture

- Library stock cultures are maintained on Sabouraud Dextrose Agar slants at 5 ± 5°C for up to 3 months prior to transfer.
- Inoculum from the library stock culture is plated over 5 plates of Sabouraud Dextrose Agar and incubated at 30°C ± 2°C for 7 to 10 days.
- After the incubation period, fungal growth is washed from the agar surface using a sterile serological pipette with an appropriate volume of sterile phosphate buffered saline solution supplemented to contain 0.1% Triton X-100. Spores are scraped off of the agar using a sterile disposable cell scraper and transferred to a sterile 50 ml conical container containing 10-20 glass beads.
- The vessel is vortex mixed thoroughly on high for approximately 2 minutes and the suspension is passed through a sterile 10-12 cc syringe containing approximately 1 cc of glass wool packed at the bottom of the syringe. The syringe is depressed to filter the suspension into a sterile conical tube labeled as the spore stock and assigned a lot number.
- The spore suspension is then enumerated by serially diluting 1:10 in sterile PBS and plated onto appropriate growth agar using standard techniques, or by counting the number of spores using a hemocytometer.
- Spore suspension may be diluted with sterile phosphate buffered saline solution or concentrated by way of centrifugation if deemed necessary to reach the required concentration of the test culture for the conduct of the study.

Supplementation of Test Culture with Organic "Soil" Load

- Thawed, sterile fetal bovine serum is added to the test culture such that the final concentration is 5% (v/v) and swirled gently to mix.

Contamination of Carriers with Test Culture

- Deionized/reverse osmosis water is aspirated from the container containing the prepared carriers using a sterile serological pipette.
- The test culture is added to the drained vessel containing the penicylinders, such that all carriers are completely submerged in the test culture for uniform coverage (Approximately 1 ml of culture per carrier).
  - The culture will completely cover the carriers. Container may be agitated to ensure all carriers are covered. A sufficient number of carriers are inoculated for the test.
- The test culture and penicylinders are allowed to stand for 15 minutes ± 2 minutes at room temperature.
- After 15 minutes ± 2 minutes have elapsed, the culture is aspirated and penicylinders are removed from the container aseptically using a sterile wire hook (carriers may be tapped or shaken prior to removal to remove excess culture) and are placed, no more than 12 carriers to a dish, on sterile double filter paper-lined, sterile Petri dishes. Carriers are placed on end, evenly spaced in the dish, such that they do not touch one another. If any carriers fall over, they are discarded from use in the efficacy testing.
- Loaded Petri dishes are covered, transferred to an incubator at 36°C ± 1°C, and allowed to dry for 40 ± 2 minutes or when visibly dry.
- Inoculated carriers are used within 2 hours of drying.

PROTOCOL (cont.)

Exposure of Carriers to Test Substance

- Inoculated carriers are transferred, using a heat sterilized wire hook, one carrier to each 25×100 mm test tube containing 10 ml test substance, at appropriate intervals to ensure careful and aseptic handling. Every attempt is made to ensure that carriers are not allowed to touch the sides of the test tube during this step. If a contaminated penicylinder touches the sides of the test tube going into the test substance, then that test tube and the corresponding subculture/neutralization test tube is noted.
- Tubes containing test substance and carrier are gently swirled then placed back in the water bath for the duration of the contact time.
- After the contact time for each carrier has elapsed, each carrier is removed from the test substance using a heat sterilized wire hook. Carriers may be tapped in the lower third of the tube to remove excess test substance. Carriers are then transferred to a tube containing 10 ml of the appropriate subculture/neutralization medium, such that it is completely submerged.
- Subculture/Neutralization tube racks are shaken and then incubated at 30°C ± 2°C for 7 days.
- After incubation, the number of subculture/neutralization tubes showing growth is recorded.

Enumeration of Test Carriers

- Following the conclusion of the dry time, carriers are assayed in two sets of three; one set immediately prior to conducting the test, and one set immediately following the test. Each carrier is transferred individually to a subculture/neutralization tube.
- These subculture/neutralization tubes are placed in a beaker, filled with water to the level of liquid in the tubes, and held by hand in a sonicator so that the beaker bottom does not touch the bottom of the sonicator. All 3 liquid levels are approximately equal, and sonicated for 1 minute ± 5 seconds, timed with a certified digital timer.
- After sonication, these subculture/neutralization tubes may be pooled for each set of three carriers, serially diluted in sterile PBS and plated in duplicate within 2 hours of sonication using standard dilution and plating techniques.
- Enumeration plates are incubated at 30°C ± 2°C for 44 to 76 hours.

Neutralization Control

- A sterile uninoculated carrier is transferred to a test tube containing 10 ml of the test substance. After the specified contact time has elapsed, the carrier is transferred to a subculture/neutralization broth tube, without allowing excess fluid to drain off of the carrier.
- After transfer, the subculture/neutralization tube is inoculated with 10-100 CFU of test microorganism (obtained by serial dilution in PBS) and incubated along with the other test materials. A parallel subculture/neutralization tube containing only neutralizer is inoculated to verify growth of the target microorganism as a comparative control.
- The inoculum is plated in duplicate to verify the number of CFU added and incubated alongside the test enumeration plates at 30°C ± 2°C for 44 to 76 hours.

Viability Control

- One to two inoculated test carriers are placed in individual subculture/neutralization broth tubes and incubated alongside the test to verify test microorganism viability.

PROTOCOL (cont.)

Subculture/Neutralization Sterility Control

- A test tube containing only subculture/neutralization broth is incubated alongside test materials to verify subculture/neutralization media sterility.

Carrier Sterility Control

- A sterile uninoculated carrier is added to a tube containing subculture/neutralization broth and is incubated alongside test materials to verify carrier sterility.

Test Microorganism Purity Control

- A volume of the test culture used in this study is subcultured to growth agar medium and incubated alongside enumeration plates to morphologically confirm presence of a pure culture.

Media Sterility Control

- A volume of PBS is added to sterile growth medium and incubated alongside enumeration plates to verify serial dilution media sterility at the time of test.
- A volume of prepared synthetic hard water is added to sterile growth medium and incubated alongside enumeration plates to verify test substance diluent sterility at the time of test.
- A volume of saline solution is added to sterile growth medium and incubated alongside enumeration plates to verify test culture diluent sterility at the time of test.
- A volume of fetal bovine serum is added to sterile growth medium and incubated alongside enumeration plates to verify organic soil sterility at the time of test.
- A plate containing only growth medium used in this study is incubated alongside enumeration plates to verify agar sterility at the time of test.

Incubation of Tubes and Enumeration and Control Plates

- All tubes are incubated for 7 days at 30°C ± 2°C
- All plates are incubated for 44-76 hours at 30°C ± 2°C

Confirmation of Positive Tubes Following Incubation

- If multiple tubes demonstrate growth, ≥20% of those tubes are confirmed not to be a result of contamination by plating on growth media, or other analysis as appropriate.
- All confirmatory plates are incubated for 44-76 hours at 30°C ± 2°C.

X. Success Criteria

The experimental success criteria follow:

- The test microorganism must demonstrate a mean log density of at least 4.0 corresponding to 1×10^4 CFU/Carrier and not above 5.0 corresponding to a mean log density of 1×10^5 CFU/Carrier.
- The media sterility control test tubes are negative for growth.

PROTOCOL (cont.)

- Viability growth control test tubes are positive for growth.
- The carrier sterility control test tubes are negative for growth.
- The neutralization control subculture/neutralization tubes are positive for growth.
- The neutralization test subculture/neutralization tubes are positive for growth.
- The neutralization control inoculum demonstrates 10-100 CFU.
- The test microorganism purity streaks shows pure culture for growth.
- Additional media sterility controls are negative for growth

The EPA performance criterion for disinfection follows:

- If 1 or more non-control subculture/neutralization test tubes are confirmed positive for *Aspergillus niger* ATCC 6275 growth after incubation, then efficacy is not demonstrated by the test substance under the conditions evaluated.

Retesting Guidance

- When a test passes and the $\log_{10}$ density of the test carriers is above 5.0, no retesting is necessary.
- When a test fails and the $\log_{10}$ density of the test carriers is below 4.0, no retesting is necessary.
- When a test passes and the $\log_{10}$ density of the test carriers is below 4.0, retesting is necessary.
- When a test fails and the $\log_{10}$ density of the test carriers is above 5.0, retesting may be conducted.

XI. Reporting

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

XII. Data and Sample Retention

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

XIII. Quality Control

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

XIV. References

- "Association of Official Analytical Chemists, Internation." AOAC Official Method 955.17. Fungicidal Activity of Disinfectants.

PROTOCOL (cont.)

Protocol for GLP AOAC Use Dilution Method Modified for Fungi
Protocol - P2108

- US EPA Product Performance Test Guidelines OCSPP 810.2200: Disinfectants for Use on hard Surfaces-- Efficacy Data Recommendations

XV. Protocol Approval

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

Study Sponsor/Representative Signature Approving Protocol

Brian Narducci, Study Sponsor, Decon 7 — Date: 3/23/18

Study Director, Microchem Laboratory, LLC — Date: 13 APR 2018

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PROTOCOL AMENDMENT

Protocol Amendment – Protocol for GLP AOAC Use-Dilution Method Modified for Fungi
Protocol - P2108
Page 1 of 1

Amendment:

The signed protocol (P2108) is hereby amended, per the testing facility, to include the following calculations used in the study. At the time of study initiation, the protocol did not specify the calculations to be used when interpreting data.

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

$$\frac{(\text{Average CFU for } 10^{-3}) + (\text{Average CFU for } 10^{-4}) + (\text{Average CFU for } 10^{-5})}{10^{-3} + 10^{-4} + 10^{-5}} = \text{CFU/ml}$$

[(CFU/ml) × 10ml] = CFU/Carrier

$$\text{Mean Log Density} = \frac{(\text{Log}_{10}\ \text{CFU/Carrier Pooled Pre Carriers} + \text{Log}_{10}\ \text{CFU/Carrier Pooled Post Carriers})}{2}$$

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

All remaining testing parameters are to be followed as stated in Protocol P2108.

Role: Study Sponsor
Name: Brian Narducci
Company: Decon 7
Address: 8541 East Anderson Drive, Suite 106, Scottsdale, AZ 85255

5/23/18
Date (dd/mmm/yyyy)

Role: Study Director
Name: Nicholas Garcia
Company: Microchem Laboratory
Address: 1304 W. Industrial Blvd, Round Rock, TX 78681

24MAY2018
Date (dd/mmm/yyyy)

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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

APPENDIX

The following were collected from efficacy testing conducted on 13APR2018. Deviations were made to adjust the neutralizer used for testing, as noted on page10 of this report.

Table A-1

The following were the carrier enumeration results for D7 (Lots: 17-391, 17-394, and 20335). Test was conducted on 13APR2018.

Test Microorganism	Test Substance	Carriers	CFU/Carrier	Log_{10} Density	Mean Log_{10} Density
A. niger ATCC 6275	D7 (Lots: 17-391, 17-394, 20335)	Pre Treatment	4.82E+04	4.68	4.71
		Post Treatment	5.50E+04	4.74	

Table A-2

The following were efficacy test results for D7 (Lots: 17-391, 17-394, 20335) when tested against *A. niger* ATCC 6275 at a contact time of 9 minutes 50 seconds ± 5 seconds. Testing conducted on 13APR2018.

Test Microorganism	Test Substance	Number of Carriers Tested	Number of Positive Subculture/Neutralizer Test Tubes
A. niger ATCC 6275	D7 (Lots: 17-391, 17-394, 20335)	10	0

Table A-3

The following were the neutralization results for D7 (Lots: 17-391, 17-394, 20335). Neutralization results were not in compliance with the aforementioned study acceptance criteria. The parallel subculture/neutralization broth control tube demonstrated no growth indicative of the target microorganism. Testing conducted on 13APR2018.

Test Microorganism	Test Substance	Plate Counts (CFU)	Average Inoculum Concentration	Neutralization Verification Result
A. niger ATCC 6275	D7 (Lots: 17-391, 17-394, 20335)	18 / 21	19.5	No Growth

APPENDIX (cont.)

Table A-4

The following were the incubation conditions for the D7 (Lots: 17-391, 17-394, 20335) test materials incubated in this study. Testing conducted on 13APR2018.

Incubation Temperature Range	Test Materials	Incubation Duration
30°C ± 2°C	Media Sterility Controls, Carrier Enumeration Plates, and Purity Streak Plate	46 Hours 51 Minute
	Test Carrier Subculture/Neutralizer Tubes, Neutralization Verification Tubes, Carrier Sterility, Viability Control, Media Sterility Tube	7 Days
	Presumptive Positive Confirmation Plates	N/A

Table A-5

The following were the incubation conditions for the D7 (Lots: 17-391, 17-394, 20335) test materials incubated in this study. Testing conducted on 13APR2018.

Test Culture	Transfer Date	Incubation Temperature Ranges	Culture Incubation Time
Initial Culture From Microbial Library Stock (Transfer 1)	22MAR2018	30 °C ± 2 °C	7 Days
Daily Transfer to SDA Plates (Transfer 2)	30MAR2018		10 Days