

FINAL STUDY REPORT

STUDY TITLE

Efficacy of a Disinfectant Applied to a Room via a Fogging, Misting or Vaporizing Device for Fungicidal Disinfection

Test Organisms:

Aspergillus niger (ATCC 6275)

PRODUCT IDENTITY

VigorOx Liquid Sanitizer and Disinfectant
Lot 03173-4 and Lot 03173-5

PROTOCOL NUMBER

PUR006071924.FRDT.1

AUTHOR

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Study Director

STUDY COMPLETION DATE

September 24, 2024

PERFORMING LABORATORY

Element Materials Technology Eagan
1285 Corporate Center Drive, Suite 110
Eagan, MN 55121

SPONSOR

Pure Maintenance LLC
334 Marshall Way, Suite D
Layton, UT 84041

PROJECT NUMBER

A39340

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: Pure Maintenance LLC

Company Agent: _____

Title

Signature

Date: _____

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.

Submitter: _____

Date: _____

Sponsor: _____

Date: _____

Study Director:  _____
Tanner Straus, B.S.Date: 9-24-24

QUALITY ASSURANCE UNIT SUMMARY

Study: Efficacy of a Disinfectant Applied to a Room via a Fogging, Misting or Vaporizing Device for Fungicidal Disinfection

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: Test substance Preparation	August 28, 2024	August 28, 2024	September 24, 2024
Final Report	September 18, 2024 September 19, 2024	September 19, 2024	

Quality Assurance Specialist: USyler Date: 09/24/24

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STUDY REPORT

GENERAL STUDY INFORMATION

Study Title: Efficacy of a Disinfectant Applied to a Room via a Fogging, Misting or Vaporizing Device for Fungicidal Disinfection

Project Number: A39340

Protocol Number: PUR006071924.FRDT.1

Sponsor: Pure Maintenance LLC
334 Marshall Way, Suite D
Layton, UT 84041

Test Facility: Element Materials Technology Eagan
1285 Corporate Center Drive, Suite 110
Eagan, MN 55121

TEST SUBSTANCE IDENTITY

Device Name: The VaPure Device

Test Substance Name: VigorOx Liquid Sanitizer and Disinfectant

Batch/Lot(s): Lot 03173-4 and Lot 03173-5

Manufacture Date(s): June 10, 2024 (Lot 03173-4)
July 1, 2024 (Lot 03173-5)

Test Substance Characterization

Test substance characterization as to identity, strength, purity, stability and uniformity, as applicable, according to 40 CFR, Part 160, Subpart F (160.105), was documented prior to its use in the study. The Test Substance Certificate of Analysis Reports may be found in Attachment I.

Test substance manufacturing information such as the manufacture and expiration date(s), chemical identity, characterization, and stability included in this report was provided by the Sponsor unless otherwise indicated. Element Materials Technology Eagan is not involved in manufacturing of the test substance(s) used in this study, and therefore, the Sponsor is responsible for ensuring the accuracy of the information for the test substance(s) used in this study.

STUDY DATES

Date Sample Received: June 13, 2024 and July 10, 2024 (Test Substance)
May 11, 2023 (Test Device)

Study Initiation Date: August 15, 2024

Experimental Start Date: August 20, 2024 (Start time: 11:05 am)

Experimental End Date: September 10, 2024 (End time: 2:30 pm)

Study Completion Date: See Page 1 of Report

OBJECTIVE

The objective of this assay was to evaluate the efficacy of a room disinfection system applied by a fogging, misting or vaporizing device for fungicidal disinfection on porous surfaces.

SUMMARY OF RESULTS

Test Substance:	VigorOx Liquid Sanitizer and Disinfectant (Lot 03173-4 and Lot 03173-5)
Dilution:	19.2 oz/gallon defined as 19.2 oz test substance + 108.8 oz sterile deionized water
Test Organisms:	<i>Aspergillus niger</i> (ATCC 6275)
Exposure Time:	60 minutes (15 minute device run time + 45 minute dwell time)
Total Cycle Time:	3 hours 56 minutes 48 seconds - 4 hours 39 minutes 51 seconds (from activation to completion of aeration)
Exposure Temperature:	Ambient temperature (20.2-23.0°C)
Exposure Humidity:	55.5 - 65.5%
Organic Soil Load:	5% fetal bovine serum
Number of Carriers:	22 per cycle/organism

Efficacy Result:

Test Organism	Test Results	
	Test Substance Name: VigorOx Liquid Sanitizer and Disinfectant	
	Lot 03173-4	Lot 03173-5
<i>Aspergillus niger</i>	Pass	Pass

Conclusion:

Test Substance	Test Organism	Efficacy Performance
VigorOx Liquid Sanitizer and Disinfectant	<i>Aspergillus niger</i>	Requirements met

TEST SYSTEM

Test System/Growth Media

Test Organism	ATCC #	Growth Medium	Incubation Parameters ²
<i>Aspergillus niger</i>	6275	Sabouraud Agar (Modified)	25-30°C, aerobic

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

²Incubation parameters listed were followed for the organism(s) during organism preparation and subculture incubation.

STUDY MATERIALS

Recovery Media

Neutralizing Subculture Medium: Sabouraud Dextrose Broth + 0.07% Lecithin + 0.5% Tween 80 + 0.01% Catalase

Agar Plate Medium: Sabouraud Dextrose Agar

Reagent(s)

Organic Soil Load Description: 5% fetal bovine serum (FBS)

Fabric Preparation

The fabric used for testing was a plain cotton weave containing approximately 80 x 80 threads/inch.

A scouring solution was prepared by adding 5.7719 grams of Na_2CO_3 and 5.8012 grams of Triton X-100 to 11.5 L of deionized water. 1154.59 grams of test fabric was added to a 11.5 L volume of scouring solution.

The solution was allowed to boil for approximately 60 minutes. The fabric was removed and rinsed by first placing the fabric into boiling water for a minimum of 5 minutes and then placing the fabric into cold water for a minimum of 5 minutes. During the rinsing procedure, the fabric was mixed in order to help remove the wetting agent. The fabric was allowed to air dry for at least 1 day ensuring that the material was completely dry.

Carrier Preparation

Fabric swatch carriers of approximately 4 inch x 1 inch were cut from the scoured fabric. Prior to sterilization, the fabric swatches were taped to clean 3 inch by 1 inch glass slides. The fabric swatch carriers were autoclave sterilized and were allowed to cool/dry at room temperature prior to use. Each carrier was transferred into a sterile Petri dish matted with 2 pieces of filter paper.

TEST METHOD

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

Preparation of Test Substance

The test substance was prepared as directed by the Sponsor as described below.

Test Substance Preparation			
Defined Dilution	Dilution Prepared		Test Substance Homogeneity Determination
	Amount Test Substance (mL)	Amount Diluent (mL)	
19.2 oz/gal defined as 19.2 oz of test substance + 108.8 oz deionized water	567.7	3217.2	Homogenous

Equipment Preparation

The prepared test substance was added to the fogging device by the sponsor. The fogging device was placed in the middle of the testing room by the sponsor.

Preparation of Test Organism(s)

A culture of *Aspergillus niger* was prepared by inoculating a sufficient number of agar plates (≥ 5 plates) using a stock culture and incubating at 25- 30°C for 10-15 days. The mycelia were removed from sufficient plates using a sterile device. The mycelia were transferred to sterile glassware containing glass beads at a ratio of 25 mL of sterile saline or saline/Triton Solution (0.85% Saline + 0.05% Triton X-100) per 5 plates harvested and agitated. The culture was filtered through sterile gauze to remove hyphal fragments. The density of the initial conidial suspension was determined by serial dilution and plating. The culture was stored at 2-8°C prior to use.

Culture Incubation Parameters	Diluent Added	Viable Cell Count	Refrigeration Time
29.0°C / 10 days	Saline/Triton Solution (0.85% Saline + 0.05% Triton X-100)	4.4×10^7 CFU/mL	11 days (8/20/2024) 18 days (8/27/2024) 19 days (8/28/2024)

The culture was adjusted to meet the carrier population control acceptance criteria. The culture was adjusted to contain 5% fetal bovine serum organic soil load. The final test culture was mixed thoroughly prior to use.

For testing performed on 8/20/2024

Test Organism	Culture Concentration		Addition of Organic Soil Load	
	Starting Volume (mL)	Final Volume (mL)	FBS Volume (mL)	Culture Volume (mL)
<i>Aspergillus niger</i>	15.0	3.0	0.10	1.90
Centrifuge Parameters	1700 (g) for 20 minutes			

For testing performed on 8/27/2024

Test Organism	Culture Concentration		Addition of Organic Soil Load	
	Starting Volume (mL)	Final Volume (mL)	FBS Volume (mL)	Culture Volume (mL)
<i>Aspergillus niger</i>	10.0	5.0	0.10	1.90
Centrifuge Parameters	1700 (g) for 20 minutes			

For testing performed on 8/28/2024

Test Organism	Culture Concentration		Addition of Organic Soil Load	
	Starting Volume (mL)	Final Volume (mL)	FBS Volume (mL)	Culture Volume (mL)
<i>Aspergillus niger</i>	8.0	4.0	0.10	1.90
Centrifuge Parameters	1700 (g) for 20 minutes			

Contamination of Carriers

The glass slide carriers were each inoculated with 10.0 μ L culture, using a calibrated pipettor uniformly spreading the culture over an approximate 1 inch x 1 inch area on the end of the slide in a Petri dish. Following inoculation, the carriers were allowed to dry for 30-32 minutes at 35-37°C (36.0-36.2°C) and 49.5-54.5% relative humidity.

Preparation of Enclosed Space

A visibly clean enclosure was used represented by the Element Materials Technology Eagan bioaerosol room. Shelves with supports, a laboratory cart and stainless steel holders were used to support applicable test carriers within the enclosure. The enclosure was appropriately sealed prior to testing in order to isolate the space in which the test substance was applied. This included confirming the seal on the exit door and all vents and ports within the enclosure. Vent dampers were used inside room enclosures to seal the standard HVAC system contained in the room.

The actual dimensions of the testing room enclosure were: 21.5' x 15' x 12' (length x width x height), totaling approximately 3870 feet³ or 110 m³, therefore a minimum of 22 carriers were required for testing, per test cycle. To meet these minimum requirements and to ensure a single carrier was placed at each carrier location, 22 carriers were evaluated, per test organism, per test cycle when using the Element Materials Technology Eagan bioaerosol room. An additional location has been added to achieve the minimum required number of test carriers. See Figure 1.

Method to Monitor the Concentration of Active Ingredient

Using Element provided PortaSense II Gas Detectors, the active ingredient was measured at the end of the device run time.

Test Date	Hydrogen Peroxide (H ₂ O ₂) Level at end of device run time	Peroxyacetic acid (PAA) Level at end of device run time
8/20/2024	12.9 ppm	44.8 ppm
8/28/2024	16.2 ppm	38.8 ppm

Carrier Placement in the Enclosed Space

Single carriers were evaluated in each of twenty-two designated locations, described in Figure 1, yielding 22 test carriers per test organism per lot of test substance. These locations were designed to evaluate the following areas of the room:

1. All corners
2. Central locations on all wall faces
3. Central locations on the floor
4. Multiple locations and heights
5. Underneath horizontal surfaces

Each carrier was oriented horizontally with the inoculum facing up. This was accomplished by placing the Petri dish containing the carrier in the enclosure with the dish lid removed.

Figure 1: Sample Diagram of Carrier Placement in a Room Enclosure

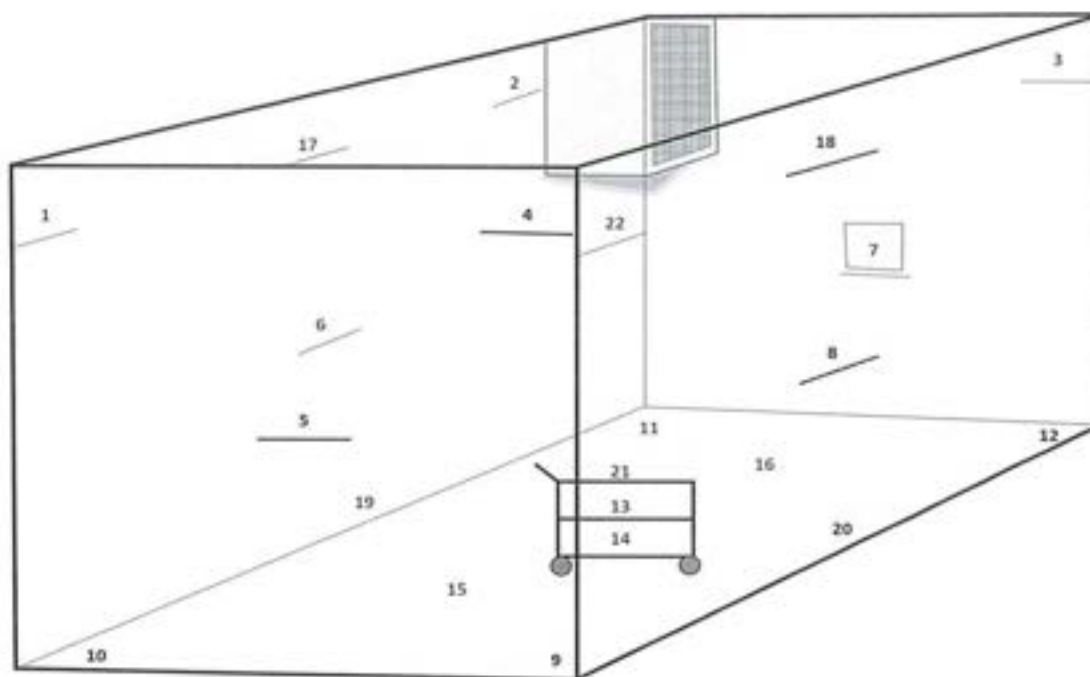


Figure 1: Illustration of a testing room, not necessarily to scale. Shelves 1-4 represent upper corners of the room. Shelves 5-8 represent central locations on all wall faces (Shelf 7 is directly under a viewing window). Spaces 9-12 represent the lower corners of the room. Spaces 13 and 14 on a 3-tier laboratory cart represent spaces underneath horizontal surfaces. Spaces 15 and 16 represent central locations very near the floor. Spaces 17 and 18 represent upper central wall faces, spaces 19 and 20 represent lower central wall faces and space 21 represents a central location on the top of the laboratory cart. Space 22 represents an upper corner beneath the testing room exhaust return. Location 22 was added to ensure 22 carriers could be evaluated in testing.

Exposure Conditions

Once the required environmental conditions were achieved (ambient temperature) and the carriers were positioned, the HVAC system and exhaust fan were turned off to ensure the vents dampers were closed. The exhaust fan was confirmed to be off. The technicians immediately exited the room. Device operation was performed by the Sponsor.

The fogging device was run for the 15 minute device run time, followed by a 45 minute dwell (exposure) time. After completion of the dwell time, the exhaust was turned on high.

For VigorOx Liquid Sanitizer and Disinfectant (Lot 03173-4), the total time from activation to the completion of cycle was 4 hours 39 minutes 51 seconds. The cycle included a 15 minute device run time, a 45 minute dwell time and a 3 hour 39 minute 51 second aeration time to assure the technicians could safely enter the room.

For VigorOx Liquid Sanitizer and Disinfectant (Lot 03173-5), the total time from activation to the completion of cycle was 3 hours 56 minutes 48 seconds. The cycle included a 15 minute device run time, a 45 minute dwell time and a 2 hour 56 minute 48 second aeration time to assure the technicians could safely enter the room.

The Element provided PortaSense II Gas Detectors was used to monitor Peroxyacetic acid and Hydrogen Peroxide to determine when it was safe to enter the testing room. The reading on the Peroxyacetic acid meter was ≤ 0.4 ppm prior to re-entry. The reading on the Hydrogen Peroxide meter was ≤ 1 ppm prior to re-entry.

Test System Recovery

After the cycle had completed, the treated carriers were individually transferred to 20 mL aliquots of neutralizing subculture medium contained in 38 mm x 100 mm tubes using sterile forceps.

Incubation and Observation

All subcultures were incubated for 44-76 hours at the incubation parameters listed in the Test System section of this report. Subcultures were stored at 2-8°C for up to three days prior to examination. The subcultures were then visually examined for the presence or absence of growth.

Test Date	Subculture Identity	Test and Control Subculture Incubation Parameters	Test and Control Subculture Refrigeration Time
8/20/2024	Subculture Plates	29.0°C/ 46.5 hours	Not Applicable
	Subculture Tubes	29.0°C/ 10 days	Not Applicable
8/27/2024	Subculture Plates	29.0°C/ 45 hours	Not Applicable
	Subculture Tubes	29.0°C/ 10 days	Not Applicable
8/28/2024	Subculture Plates	29.0°C/ 45 hours	Not Applicable
	Subculture Tubes	29.0°C/ 10 days	3 days

STUDY CONTROLS

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

Purity Control

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

Organic Soil Sterility Control

The serum used for the organic soil load was cultured, incubated, and visually examined for lack of growth. The acceptance criterion for this study control is lack of growth.

Neutralizing Subculture Medium Sterility Control

A representative sample of each uninoculated neutralizing subculture medium was incubated and visually examined. The acceptance criterion for this study control is lack of growth.

Viability Control

One representative inoculated carrier, per test organism, was added to a vessel containing subculture medium. The vessels containing each carrier were incubated and visually examined for growth. The acceptance criterion for this study control is growth in the subculture medium.

Carrier Sterility Control

A representative carrier used in testing was added to a tube of neutralizing subculture medium, incubated and visually examined. The acceptance criterion for this study control is lack of growth.

Initial Population Control

A control was performed to assess the level of test organism present immediately following drying. Three inoculated carriers for each organism were individually assayed just after drying.

Each inoculated carrier was subcultured into 20 mL of neutralizing broth and immediately vortex mixed for 120±5 seconds. Following mixing, the contents of the three subcultured carriers, per test organism, were pooled (60 mL). Appropriate serial ten-fold dilutions were prepared and the duplicate 0.100 mL aliquots of the 10⁰ through 10⁻⁴ dilutions were spread plated on the recovery agar plate medium, and incubated.

Following incubation, the resulting colonies were enumerated and the CFU per carrier calculated. The individual CFU per carrier set results were calculated, and the Log₁₀ value of each carrier set determined. The acceptance criterion for this study control is a minimum Log₁₀ value of 4.0.

Carrier Population Control (post-testing)

In order to ensure the minimum carrier population load required for valid disinfection was present at the end of the test cycle, a carrier population control was performed just after the completion of each test cycle. Three inoculated carriers for each organism were individually assayed just after each test cycle was completed by holding the dried carriers at room temperature outside of the testing room.

Each inoculated carrier was subcultured into 20 mL of neutralizing broth and immediately vortex mixed for 120 ± 5 seconds. Following mixing, the contents of the three subcultured carriers, per test organism, were pooled (60 mL). Appropriate serial ten-fold dilutions were prepared and the duplicate 0.100 mL aliquots of the 10^0 through 10^{-4} dilutions were spread plated on the recovery agar plate medium, and incubated.

Following incubation, the resulting colonies were enumerated and the CFU per carrier calculated. The individual CFU per carrier set results were calculated, and the $\log_{10}$ value of each carrier set determined. The acceptance criterion for this study control is a minimum $\log_{10}$ value of 4.0.

Non-Active Treatment Control

A control was performed to evaluate the reduction of the test organism after exposure to sterile deionized water. In a separate test cycle, the room was prepared in a manner consistent with the test cycles.

A total of three inoculated and dried carriers, per test organism, were evaluated in this control at locations representative of the test cycle. The first carrier was placed at location #1, the second carrier was placed at location #7 and the third carrier was placed at location #15 to represent each orientation at high, mid and low locations around the room. All carriers were oriented horizontally with the inoculum facing upward to simulate the test cycle orientations (Refer to figure 1).

The carriers were exposed to the non-active solution in a manner consistent with the intended test cycle parameters at $20.2-21.2^\circ\text{C}$ and 60.4-80.7% relative humidity. The total cycle time from activation through completion of aeration was 5 hours and 52 minutes. After the completion of the cycle, each inoculated carrier was subcultured into 20 mL of neutralizing broth and immediately vortex mixed for 120 ± 5 seconds.

Following mixing, appropriate serial ten-fold dilutions were prepared for each individual carrier and duplicate 0.100 mL aliquots of the 10^0 through 10^{-4} dilutions were spread plated on the recovery agar plate medium, and incubated. Following incubation, the resulting colonies were enumerated and the CFU per carrier calculated. The individual CFU per carrier results were calculated, and the $\log_{10}$ value of each carrier was determined. The acceptance criterion for the non-active treatment control is a minimum $\log_{10}$ value of 3.0 for each test organism.

Neutralization Confirmation Control

Concurrent with testing, a neutralization confirmation control was performed. More than one organism dilution was used in this control.

The neutralization of the test substance active was confirmed by exposing sterile carriers to the test substance. To represent worst-case scenario, these carriers were oriented in a horizontal position during exposure and were placed next to the test device application.

Following exposure, the carriers were transferred to subcultures containing 20 mL of neutralizing subculture medium as in the test assay. The subcultures were inoculated with a target of 10-100 colony forming units (CFU) of each test organism, incubated under test conditions and visually examined for the presence of growth. A standardized spread plate procedure was performed concurrently in order to enumerate the number of CFU actually added by plating identical aliquots, in duplicate, onto the recovery agar medium. The acceptance criterion for this study control is growth in the subculture broth following inoculation with ≤ 100 CFU.

STUDY ACCEPTANCE CRITERIA

Test Substance Performance Criteria

In order to be considered effective for fungicidal efficacy, the test substance must kill the organism on all 22 carriers.

Control Acceptance Criteria

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

PLANNED PROTOCOL CHANGES

Protocol Amendment(s):

1. Per sponsor request, the protocol is amended to change the device run time from 30 minutes to 15 minutes.
2. The protocol is amended for the following:
 - a. Per sponsor request, the protocol is amended to change the number of supplemental fan(s) to be used in testing in the Additional Testing Room Setup Requirements section on page 11 of the protocol from 1 to 0.
 - b. Due to typographical error, the protocol is amended to change the reading of peroxyacetic acid for Safe re-entry into the room on page 12 of the protocol from ≤ 4 ppm to ≤ 0.4 ppm.
3. The protocol is amended for the following:
 - a. Due to typographical error, page 2 of attachment I is changed from "a single carrier will be evaluated in each of twenty designated locations" to "a single carrier will be evaluated in each of twenty-two designated locations".
 - b. Due to typographical error, page 3 of attachment I is updated to remove reference #3 because it is a duplicate of reference #2.

- c. Due to typographical error, the proposed completion date on page 2 of the protocol is updated from "August 16, 20924" to "August 16, 2024".
- d. Due to typographical error, the Sponsor address on protocol amendments 1-2 are corrected to the following:

Pure Maintenance LLC
334 Marshall Way, Suite D
Layton, UT 84041

Protocol Deviation(s):

The protocol states to prepare the organism "the conidial concentration will be estimated by counting in a hemacytometer". The fungal suspension used for all three test dates had the concentration estimated by plating the fungal suspension on agar plates. Since the initial suspension was still able to be determined and the carrier population controls met the acceptance criteria before and after testing, there is no impact on the overall intent of the protocol.

UNFORESEEN CIRCUMSTANCES

No unforeseen circumstances occurred during this study.

DATA ANALYSIS

Calculations

The CFU/carrier in the Initial Population Control, Carrier Population Control (post-testing) and non-active treatment control was determined using all average counts between 0-300 CFU as follows:

$$\text{CFU/carrier} = \frac{[(\text{avg. CFU for } 10^{-x}) + (\text{avg. CFU for } 10^{-y}) + (\text{avg. CFU for } 10^{-z})] \times (\text{Volume of neutralizer})}{[10^{-x} + 10^{-y} + 10^{-z}] \times (\text{Volume plated}) \times (\# \text{ of carriers per set})}$$

where 10^{-x} , 10^{-y} , and 10^{-z} are example dilutions that may be used

Statistical Analysis

None used.

STUDY RETENTION

Record Retention

All of the original raw data developed exclusively for this study shall be archived at Element Materials Technology Eagan following the record retention policy outlined in the internal SOP ALS-0032. The original data includes, but is not limited to, the following:

REFERENCES

1. Association of Official Analytical Chemists (AOAC) Official Method 955.17, Fungicidal Activity of Disinfectants. In Official Methods of Analysis of the AOAC, 1955 Edition.
2. U.S. Environmental Protection Agency, Registration Division, Office of Pesticide Programs, Draft Protocol "Protocol for Sterilization of Porous and Non-Porous Surfaces within Sealed Enclosures using Vaporized Hydrogen Peroxide".
3. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, OCSPP 810.2000: General Considerations for Testing Public Health Antimicrobial Pesticides – Guidance for Efficacy Testing, February 2018.
4. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, OCSPP 810.2200: Disinfectants for Use on Environmental Surfaces – Guidance for Efficacy Testing, February 2018.
5. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, Series 810 Guidelines FAQ, August 2019.
6. U.S. Environmental Protection Agency, Office of Pesticide Programs SOP Number: MB-30-03, Preparation of Hard Water and Other Diluents for Preparation of Antimicrobial Products, November 14, 2022.

RESULTS

For Control and Neutralization Results, see Tables 1-4.

All data measurements including the culture purity, organic soil sterility, viability, neutralizing subculture medium sterility, carrier sterility, initial population, carrier population, non-active treatment control and neutralization confirmation controls were within acceptance criteria.

For Test Results, see Table 5.

Key for Control and Results Tables:

CFU = Colony forming unit(s)

T = Colonies were too numerous to count (>300 colonies)

ANALYSIS

The results of VigorOx Liquid Sanitizer and Disinfectant (Lot 03173-4 and Lot 03173-5) diluted 19.2 oz/gal defined as 19.2 oz of test substance + 108.8 oz deionized water, exposed to and *Aspergillus niger* (ATCC 6275), in the presence of a 5% fetal bovine serum organic soil load at room temperature (20.2-23.0°C) and 55.5-65.5% relative humidity for a 15 minute device run time followed by a 45 minute dwell time (3 hours 56 minutes 48 seconds - 4 hours 39 minutes 51 seconds total cycle time) were as follows:

Lot 03173-4 demonstrated no growth of *Aspergillus niger* in any of the 22 subcultures.

Lot 03173-5 demonstrated no growth of *Aspergillus niger* in any of the 22 subcultures.

STUDY CONCLUSION

Under the conditions of this investigation, VigorOx Liquid Sanitizer and Disinfectant, diluted 19.2 oz/gal defined as 19.2 oz of test substance + 108.8 oz deionized water, demonstrated efficacy against *Aspergillus niger* as required by the U.S. EPA following a 15 minute device run time and 45 minute dwell time (3 hours 56 minutes 48 seconds - 4 hours 39 minutes 51 seconds total cycle time) when tested at room temperature (20.2-23.0°C) and 55.5-65.5% relative humidity.

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

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TABLE 1: CONTROL RESULTS

The following results from controls confirmed study validity:

Test Date	Type of Control	Results
		<i>Aspergillus niger</i> (ATCC 6275)
8/20/2024	Purity Control	Pure
	Viability Control	Growth
	Organic Soil Sterility	No Growth
	Carrier Sterility Control	No Growth
	Neutralizing Subculture Medium Sterility Control	No Growth
8/27/2024	Purity Control	Pure
	Viability Control	Growth
	Organic Soil Sterility	No Growth
	Carrier Sterility Control	No Growth
	Neutralizing Subculture Medium Sterility Control	No Growth
8/28/2024	Purity Control	Pure
	Viability Control	Growth
	Organic Soil Sterility	No Growth
	Carrier Sterility Control	No Growth
	Neutralizing Subculture Medium Sterility Control	No Growth

**TABLE 2: INITIAL POPULATION & CARRIER POPULATION CONTROL
(POST-TESTING) RESULTS**

Test Date: 8/20/2024, Lot 03173-4							
Test Organism: <i>Aspergillus niger</i> (ATCC 6275)							
Volume Plated: 0.100 mL							
Carrier set	Dilution Factor					CFU/ carrier	Log ₁₀
	10 ⁰	10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻⁴		
Initial Population	T, T	61, 64	11, 11	3, 1	0, 0	1.4 x 10 ⁵	5.15
Post-testing	T, T	46, 53	10, 5	0, 0	0, 0	1.0 x 10 ⁵	5.00
Test Date: 8/28/2024, Lot 03173-5							
Test Organism: <i>Aspergillus niger</i> (ATCC 6275)							
Volume Plated: 0.100 mL							
Carrier set	Dilution Factor					CFU/ carrier	Log ₁₀
	10 ⁰	10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻⁴		
Initial Population	T, T	39, 29	2, 0	0, 0	0, 0	6.3 x 10 ⁴	4.80
Post-testing	T, T	39, 44	7, 1	1, 1	0, 0	8.5 x 10 ⁴	4.93

TABLE 3: NON-ACTIVE TREATMENT CONTROL RESULTS

Test Organism: <i>Aspergillus niger</i> (ATCC 6275)						
Volume Plated: 0.100 mL						
Dilution Factor					CFU/ carrier	Log ₁₀
10 ⁰	10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻⁴		
48, 55	14, 14	2, 1	0, 0	0, 0	1.2 x 10 ⁴	4.08

TABLE 4: NEUTRALIZATION CONFIRMATION CONTROL RESULTS

Test Substance	Test Organism	Dilution	CFU Added	Average CFU	Subculture Tube Results (One Tube Inoculated per Dilution)
VigorOx Liquid Sanitizer and Disinfectant, Lot 03173-4	<i>Aspergillus niger</i> (ATCC 6275)	10 ⁻⁴	T, T	>300	Growth
		10 ⁻⁵	35, 41	38	Growth
		10 ⁻⁶	6, 4	5	Growth
VigorOx Liquid Sanitizer and Disinfectant, Lot 03173-5	<i>Aspergillus niger</i> (ATCC 6275)	10 ⁻⁴	T, T	>300	Growth
		10 ⁻⁵	53, 71	62	Growth
		10 ⁻⁶	9, 14	12	Growth

TABLE 5: TEST RESULTS

Test Substance	Test Organism	Test Cycle time	Number of Carriers	
			Exposed	Showing Growth*
VigorOx Liquid Sanitizer and Disinfectant, Lot 03173-4	<i>Aspergillus niger</i> (ATCC 6275)	15 minute device run time 45 minute dwell	22	0
VigorOx Liquid Sanitizer and Disinfectant, Lot 03173-5	<i>Aspergillus niger</i> (ATCC 6275)	15 minute device run time 45 minute dwell	22	0

* Number of carriers showing growth of the test organism.

ATTACHMENT I: TEST SUBSTANCE CERTIFICATE OF ANALYSIS
Lot 03173-4



FOR CUSTOMER SERVICE PLEASE CALL: 1-855-850-4760

Certificate of Analysis

Shipped to: Element Eagan 1285 Corporate Center Drive Suite 110 Eagan, MN 55121	Evonik Order # Customer PO #	SAMPLE SAMPLE
Ship Date: 10-Jun-24	Certificate Date: 10-Jun-24	

Product: VigorOx 5%

Property Inspection Method	Specification	BATCH/Mfg.Dt 03173-4 10-Jun-24	BATCH/Mfg.Dt	BATCH/Mfg.Dt
Assay, % QC0729	4.70% Min.	4.75		
Hydrogen Peroxide, % QC0729	19.70% Min.	19.72		

Authorized By:

[Internal]

TEST SUBSTANCE CERTIFICATE OF ANALYSIS
Lot 03173-5



FOR CUSTOMER SERVICE PLEASE CALL: 1-866-800-4760

Certificate of Analysis

Shipped to: Element Eagan 1285 Corporate Center Drive Suite 110 Eagan, MN 55121	Evonik Order # Customer PO #	SAMPLE SAMPLE
Ship Date: 05-Jul-24	Certificate Date: 01-Jul-24	

Product: VigorOx 5%

Property Inspection Method	Specification	BATCH/Mfg.Dt 03173-5 01-Jul-24	BATCH/Mfg.Dt 03173-6 01-Jul-24	BATCH/Mfg.Dt
Assay, % QC0729	4.70% Min.	4.75	4.75	
Hydrogen Peroxide, % QC0729	19.70% Min.	19.81	19.72	

Authorized By:

[Internal]



AMENDMENT TO GLP TEST PROTOCOL

Amendment No.: 3

Effective Date: September 18, 2024

Sponsor: Pure Maintenance LLC
334 Marshall Way, Suite D
Layton, UT 84041

Test Facility: Element Materials Technology Eagan
1285 Corporate Center Drive, Suite 110
Eagan, MN 55121

Protocol Title: Efficacy of a Disinfectant Applied to a Room via a Fogging,
Misting or Vaporizing Device for Fungicidal Disinfection

Protocol Number: PUR006071924.FRDT.1

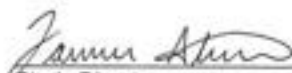
Project Number: A39340

Modifications to Protocol:

The protocol is amended for the following:

- Due to typographical error, page 2 of attachment I is changed from "a single carrier will be evaluated in each of twenty designated locations" to "a single carrier will be evaluated in each of twenty-two designated locations".
- Due to typographical error, page 3 of attachment I is updated to remove reference #3 because it is a duplicate of reference #2.
- Due to typographical error, the proposed completion date on page 2 of the protocol is updated from "August 16, 20924" to "August 16, 2024".
- Due to typographical error, the Sponsor address on protocol amendments 1-2 are corrected to the following:

Pure Maintenance LLC
334 Marshall Way, Suite D
Layton, UT 84041


Study Director

9-24-24
Date



AMENDMENT TO GLP TEST PROTOCOL

Amendment No.: 2

Effective Date: August 20, 2024

Sponsor: Pure Maintenance LLC
344 Marshall Way, Suite D
Layton, UT 84041

Test Facility: Element Materials Technology Eagan
1285 Corporate Center Drive, Suite 110
Eagan, MN 55121

Protocol Title: Efficacy of a Disinfectant Applied to a Room via a Fogging,
Misting or Vaporizing Device for Fungicidal Disinfection

Protocol Number: PUR006071924.FRDT.1

Project Number: A39340

Modifications to Protocol:

The protocol is amended for the following:

- Per sponsor request, the protocol is amended to change the number of supplemental fan(s) to be used in testing in the Additional Testing Room Setup Requirements section on page 11 of the protocol from 1 to 0.
- Due to typographical error, the protocol is amended to change the reading of peroxyacetic acid for Safe re-entry into the room on page 12 of the protocol from ≤ 4 ppm to ≤ 0.4 ppm.


Study Director

8-21-24
Date



AMENDMENT TO GLP TEST PROTOCOL

Amendment No.: 1

Effective Date: August 16, 2024

Sponsor: Pure Maintenance LLC
344 Marshall Way, Suite D
Layton, UT 84041

Test Facility: Element Materials Technology Eagan
1285 Corporate Center Drive, Suite 110
Eagan, MN 55121

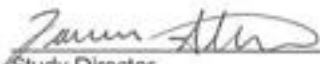
Protocol Title: Efficacy of a Disinfectant Applied to a Room via a Fogging,
Misting or Vaporizing Device for Fungicidal Disinfection

Protocol Number: PUR006071924.FRDT.1

Project Number: A39340

Modifications to Protocol:

Per sponsor request, the protocol is amended to change the device run time from 30 minutes to 15 minutes.


Study Director

8-19-24
Date

For Laboratory Use Only		
Quote #: PUR006071724AM	Quote #: PUR006071924-1	Quote #: PUR006071924-2
Element Materials Technology Eagan Project		
Test Substance Tracking #: T5071924-010006	Test Substance Tracking #: T5071924-010007	Test Substance Tracking #: T5071924-010008
T5071924-010006	T5071924-010007	T5071924-010008

A39340



PROTOCOL

Efficacy of a Disinfectant Applied to a Room via a Fogging, Misting or Vaporizing Device for Fungicidal Disinfection

Test Organism(s):

Aspergillus niger (ATCC 6275)

PROTOCOL NUMBER

PUR006071924.FRDT.1

SPONSOR

Pure Maintenance LLC
334 Marshall Way, Suite D
Layton, UT 84041

PERFORMING LABORATORY

Element Materials Technology Eagan
1285 Corporate Center Drive, Suite 110
Eagan, MN 55121

DATE

July 19, 2024

Efficacy of a Disinfectant Applied to a Room via a Fogging, Misting or Vaporizing Device for Fungicidal Disinfection

PURPOSE

The purpose of this assay is to evaluate the efficacy of a room disinfection system applied by a fogging, misting or vaporizing device for fungicidal disinfection on hard, non-porous surfaces.

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 Element Materials Technology Eagan. Element Materials Technology Eagan will append Sponsor-provided Certificates of Analysis (C of A) to this study report, if requested and supplied. Characterization and stability studies not performed by or under the direction of Element Materials Technology Eagan 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 Element Materials Technology Eagan receives the Sponsor approved/completed protocol, signed fee schedule and corresponding test substance(s). Based on all required materials being received at this time, the proposed experimental start date is August 2, 2024. Verbal results may be given upon completion of the study with a written report to follow on the proposed completion date of August 16, 2024. To expedite scheduling, please be sure all required paperwork and test substance documentation is complete/accurate upon arrival at Element Materials Technology Eagan.

If a test must be repeated, or a portion of it, due to failure by Element Materials Technology Eagan 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 Element Materials Technology Eagan nor any of its employees are to be used in advertising or other promotion without written consent from Element Materials Technology Eagan. The Sponsor is responsible for any rejection of the final report by the regulating agencies concerning report format, pagination, etc. To prevent rejection, Sponsor should carefully review the Element Materials Technology Eagan final report and notify Element Materials Technology Eagan of any perceived deficiencies in these areas before submission of the report to the regulatory agency. Element Materials Technology Eagan 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 specific disinfection claims for disinfectant products and bio-decontamination systems intended for use on hard surfaces be supported by appropriate scientific data demonstrating the efficacy of the product and delivery system against the claimed test organism. Testing is accomplished in the laboratory by treating the test organism with the test substance under conditions which simulate as closely as possible the actual conditions under which the test substance is designed to be used. For fogging or misting bio-decontamination devices which utilize a disinfectant, efficacy testing is performed to determine that all exposed, hard, non-porous surfaces within the enclosure are effectively treated with the disinfectant. This protocol has been reviewed by the U.S. EPA and determined to be adequate for testing the efficacy of a disinfectant on hard, non-porous surfaces in a room treated via a fogging, misting or vaporizing device (MRID 493679-01). Fungicidal disinfection claims can be made only after disinfection of the required organisms (e.g. *S. aureus*, *S. enterica* and/or *P. aeruginosa*) has been achieved. Additional field studies not addressed herein may also be required by regulatory agencies for registration.

TEST PRINCIPLE

Inoculated glass carriers and appropriate chemical indicators or active ingredient detection probes are placed at diverse locations within the sealed room. The inoculated carriers and indicators/detection probes will be exposed to the test substance for a specified exposure time. After exposure, the carriers are transferred to vessels containing neutralizing subculture media and assayed for survivors. Appropriate culture purity, sterility, viability, initial population, carrier population, non-active treatment and neutralization confirmation controls will be performed. The current revision of Standard Operating Procedure CGT-0026 reflects the methods which shall be used in this study.

TEST METHOD

Table 1:

Test Organism	Designation #	Growth Medium	Incubation Parameters
<i>Aspergillus niger</i>	6275	Sabouraud Agar (Modified)	25-30°C, aerobic

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

Neutralizing Subculture Medium: Appropriate to neutralize the test substance active ingredient and recover the test organism.

Recovery Agar: Sabouraud Dextrose Agar

Preparation of Test Organism

The *Aspergillus niger* conidial suspension will be prepared by inoculating a flask of Sabouraud agar (Modified) (aka Neopeptone agar) and incubating for 7-10 days at 25-30°C. Following incubation, sterile saline or saline/Triton Solution (0.85% Saline + 0.05% Triton X-100) and sterile glass beads are added to the flask. The flask is agitated to remove mycelia/conidia from the agar. The conidia suspension is aspirated from the flask and passed through sterile gauze to remove hyphal fragments. The conidial concentration is estimated by counting in a hemacytometer and the culture may be adjusted as necessary. Cultures may be refrigerated up to 4 weeks prior to use. An organic soil load will be added to the test culture per Sponsor's request. The final test culture will be mixed thoroughly prior to use.

Carriers

To represent non-porous surfaces and to ensure compatibility with the placement of the surfaces in the testing room, three inch by one inch clean glass microscope slides will be utilized as the carriers in this study. Clean slides will be steam sterilized or sterilized in a hot air oven at ≥180°C for ≥2 hours. One sterile glass slide will be transferred into a sterile Petri dish matted with 2 pieces of filter paper.

Contamination of Carriers

The glass slide carriers will each be inoculated with 10 µL culture, using a 4 mm loop or calibrated pipettor uniformly spreading the culture over an approximate 1 inch x 1 inch area on the end of the slide in a Petri dish. Following inoculation, the carriers will be dried for 30-40 minutes at 35-37°C.

Preparation of Enclosed Space

A visibly clean enclosure will be used. Depending on the application, this may be represented by an entire room or large chamber. Shelves with supports, laboratory carts or other suitable means of supporting the test carriers will be positioned within the enclosure. The enclosure will be appropriately sealed prior to testing in order to isolate the space in which the test substance will be applied. At a minimum, this will include confirming the seal on exit doors and any vents or ports within the enclosure. Vent dampers are used inside room enclosures to seal the standard HVAC system contained in the room. A humidifier or dehumidifier may be used, by Sponsor request, to reach the desired environmental conditions. In addition, supplemental fans may be used to assist with distribution of the test substance at the Sponsor's request.

For supplemental organisms including fungi, a single carrier at a minimum of twenty designated locations, totaling 20 test carriers per test organism, are required to substantiate efficacy claims. The locations that have been selected are designed to evaluate the extremes of the enclosure. Refer to figure 1 for an example of a room design that may be used.

The actual volume of the testing enclosure will be reported.

Method to Monitor the Concentration of Active Ingredient

To ensure the minimum concentration of active is delivered to the room, the Sponsor is responsible for providing a method to monitor the level of active ingredient present in the room during each test cycle. Chemical indicators or detection device(s) may be used. At minimum, an indicator or detection device/probe will be placed at a location representing where the concentration of active is expected to be the lowest (worst-case condition). The detected level of active will be reported. If a detection method is not available to detect the specific active ingredient, the Sponsor is responsible for providing justification as to the level of active ingredient applied in each test cycle.

Test Substance and Equipment Preparation

The test substance and device to be assayed will be used as directed by the Sponsor. The appropriate volume of test substance will be added to the Sponsor provided device and the device will be activated per Sponsor's instruction.

Carrier Placement in the Enclosed Space

For all supplemental organisms including fungi, for each batch of test substance, a single carrier will be evaluated in each of twenty designated locations, described in figure 1, yielding 20 test carriers per test organism. These locations are designed to evaluate the following areas of the room:

1. All corners
2. Central locations on all wall faces
3. Central locations on the floor
4. Multiple locations and heights
5. Underneath horizontal surfaces

Each carrier will be oriented horizontally with the inoculum facing up. This will be accomplished by placing the Petri dish containing the carrier in the enclosure with the dish lid removed.

Figure 1: Sample Diagram of Carrier Placement in a Room Enclosure

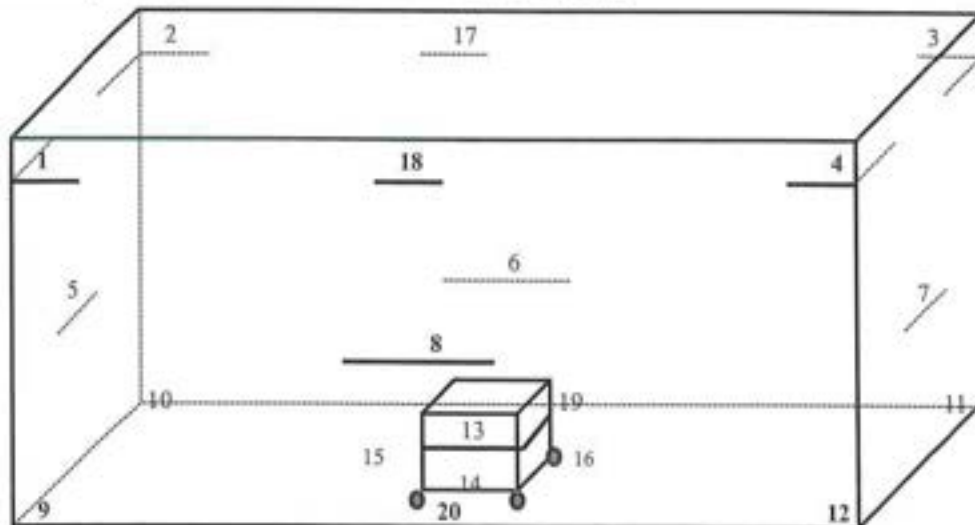


Figure 1: Illustration of the testing room is not necessarily to scale. Shelves 1-4 represent upper corners of the room. Shelves 5-8 represent central locations on all wall faces. Spaces 9-12 represent the lower corners of the room. Spaces 13 and 14 on a 3-tier laboratory cart represent spaces underneath horizontal surfaces. Spaces 15 and 16 represent central locations on or near the floor. Spaces 17 & 18 represent upper central wall faces and spaces 19 and 20 represent lower central wall faces on or near the floor.

Exposure Conditions

Template: 247-1 Rev. 003B

— Proprietary Information —

1285 Corporate Center Drive, Suite 110 • Eagan, MN 55121 • 877.287.8378 • 651.379.5510 • <https://www.element.com>

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Once the required environmental conditions have been achieved and the carriers have been positioned, the HVAC system and exhaust fan will be turned off to ensure the vents dampers are closed. The exhaust fan will be confirmed to be off.

The test device will be activated per Sponsor's direction and the technician will immediately exit the room.

The cycle will be allowed to continue until completion. The total time from activation to the completion of cycle will be documented.

The Sponsor will be responsible for ensuring there is a mechanism in place to confirm safe re-entry into the room. In addition, the room exhaust will be turned on high (clearing about 1000 cubic feet per minute or 28 cubic meters per minute) for at least 8 more minutes prior to entry. This will clear the room at least 2 times.

Test System Recovery

After the cycle has been completed, the treated carriers will be individually transferred to 20 mL aliquots of neutralizing subculture medium contained in 38 mm x 100 mm tubes using sterile forceps.

If neutralization is of concern, carriers may be transferred into individual secondary subcultures containing 20 mL aliquots of neutralizing subculture medium approximately 25-60 minutes after primary transfer.

The results of the chemical indicators or detection probes will be documented and reported.

Incubation and Observation

Incubate the subculture plates at 25-30°C for a minimum of 44-76 hours.

Incubate the broth subcultures at 25-30°C for 10 days or 2-3 days (*C. albicans*).

If necessary, the subcultures may be refrigerated at 2-8°C for up to three days prior to examination. Following incubation, the subcultures will be visually examined for growth and subculture plates will be enumerated, as applicable. Representative test subcultures showing growth will be subcultured, stained and/or biochemically assayed to confirm or rule out the presence of the test organism.

STUDY CONTROLS

Purity Control

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

Organic Soil Sterility Control

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

Neutralizing Subculture Medium Sterility Control

Prior to or concurrent with testing, a representative sample of uninoculated neutralizing subculture medium will be incubated and visually examined. The acceptance criterion for this study control is lack of growth.

Viability Control

One representative inoculated carrier will be added to a vessel containing each type of subculture medium. If secondary subcultures are performed using a different media type, one carrier will be placed in the primary subculture medium and one carrier will be placed in the secondary subculture medium. The vessels containing each carrier will be incubated and visually examined for growth. The acceptance criterion for this study control is growth in the subculture media.

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Carrier Sterility Control

Prior to or concurrent with testing, a representative carrier used in testing will be added to a tube of neutralizing subculture medium, will be incubated and visually examined. The acceptance criterion for this study control is lack of growth.

Initial Population Control

A control will be performed to assess the level of test organism present immediately following drying. Three inoculated carriers for each organism will be individually assayed just after drying. This control may be evaluated (processed) concurrent with test cycle exposure.

Each inoculated carrier will be subcultured into 20 mL of neutralizing broth and immediately vortex mixed for 120±5 seconds.

Following mixing, the contents of the three subcultured carriers, per test organism, will be pooled (60 mL). Appropriate serial ten-fold dilutions will be prepared and the duplicate 0.1 mL aliquots of the 10^0 through 10^{-4} dilutions will be spread plated on the recovery agar plate medium, and incubated. If serial dilutions are not performed and plated immediately following mixing, the vessels may be refrigerated at 2-8°C for up to 2 hours prior to dilution.

Following incubation, the resulting colonies will be enumerated and the CFU per carrier calculated. The individual CFU per carrier set results will be calculated, and the $\log_{10}$ value of each carrier set determined. The acceptance criterion for this study control is a minimum $\log_{10}$ value of 4.0.

Carrier Population Control (post-testing)

In order to ensure the minimum carrier population load required for valid disinfection was present at the end of the test cycle, a carrier population control will be performed just after the completion of each test cycle.

Three inoculated carriers for each organism will be individually assayed just after each test cycle is completed by holding the dried carriers at room temperature outside of the testing room or enclosure.

Each inoculated carrier will be subcultured into 20 mL of neutralizing broth and immediately vortex mixed for 120±5 seconds.

Following mixing, the contents of the three subcultured carriers, per test organism, will be pooled (60 mL). Appropriate serial ten-fold dilutions will be prepared and the duplicate 0.1 mL aliquots of the 10^0 through 10^{-4} dilutions will be spread plated on the recovery agar plate medium, and incubated. If serial dilutions are not performed and plated immediately following mixing, the vessels may be refrigerated at 2-8°C for up to 2 hours prior to dilution.

Following incubation, the resulting colonies will be enumerated and the CFU per carrier calculated. The individual CFU per carrier set results will be calculated, and the $\log_{10}$ value of each carrier set determined. The cycle parameters (including length of time) must be carefully considered to ensure this control meets the designated acceptance criterion. The acceptance criterion for this study control is a minimum $\log_{10}$ value of 4.0.

Non-Active Treatment Control

A control will be performed to evaluate the reduction of the test organism after exposure to a Sponsor-provided formulation containing no active ingredient. If such a formulation cannot be provided, sterile deionized water may be used in its place. It is recommended that this control be performed prior to testing to ensure the acceptance criterion can be met. The test cycle parameters (including length of time) must be carefully considered to ensure this control meets the designated acceptance criterion.

In a separate test cycle, the room will be prepared in a manner consistent with the test cycles.

A total of three inoculated and dried carriers, per test organism, will be evaluated in this control at locations representative of the test cycle. The first carrier will be placed at location #1, the second carrier will be placed at location #7 and the third carrier will be placed at location #15 to represent each orientation at high, mid and low locations around the room. (Refer to figure 1.)

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All carriers will be oriented horizontally with the inoculum facing upward to simulate the test cycle orientations. The actual locations and orientations will be documented and reported.

The carriers will be exposed to the non-active solution in a manner consistent with the intended test cycle parameters.

After the completion of the cycle, each inoculated carrier will be subcultured into 20 mL of neutralizing broth and immediately vortex mixed for 120±5 seconds. Following mixing, the contents of the three subcultured carriers, per test organism, will be pooled (60 mL).

Appropriate serial ten-fold dilutions will be prepared and the duplicate 0.1 mL aliquots of the 10^2 through 10^{-4} dilutions will be spread plated on the recovery agar plate medium, and incubated. If serial dilutions are not performed and plated immediately following mixing, the vessels may be refrigerated at 2-8°C for up to 2 hours prior to dilution. Following incubation, the resulting colonies will be enumerated and the CFU per carrier calculated. The individual CFU per carrier set results will be calculated, and the $\log_{10}$ value of each carrier set determined.

The acceptance criterion for the non-active treatment control is a minimum $\log_{10}$ value of 3.0.

Neutralization Confirmation Control

Concurrent with testing or prior to testing, a neutralization confirmation control will be performed. More than one organism dilution should be used in this control. If all the organism dilution(s) used in this control fail to provide adequate numbers (10-100 CFU) which coincides in a failure to meet the acceptance criterion for this study control, the control may be repeated in its entirety. Only the most concentrated test substance dilution and/or longest exposure time needs to be evaluated in this control.

The neutralization of the test substance active will be confirmed by exposing sterile carriers to the test substance. To represent worst-case scenario, these carriers will be oriented in a horizontal position during exposure and will be placed at sites near the test device application site.

Following exposure, the carriers will be transferred to primary subcultures containing 20 mL of neutralizing subculture medium as in the test assay. Carriers may be transferred from primary subcultures into individual secondary subcultures beginning approximately 25-60 minutes following the primary transfer.

The subcultures will be inoculated with a target of 10-100 colony forming units (CFU) of each test organism, incubated under test conditions and visually examined for the presence of growth. A standardized spread plate procedure will be performed concurrently in order to enumerate the number of CFU actually added.

The acceptance criterion for this study control is growth in the final subculture broth, minimally, following inoculation with ≤ 100 CFU.

PROCEDURE FOR IDENTIFICATION OF THE TEST SYSTEM

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

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

Protocol Number: PUR006071924.FRDT.1

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STUDY ACCEPTANCE CRITERIA

Test Substance Performance Criteria

In order to be considered effective for fungicidal efficacy, the test substance must kill the test organism on all 20 inoculated carriers.

Control Acceptance Criteria

The study controls must perform according to the criteria detailed in the study controls description section. If any of the control acceptance criteria are not met, the test may be repeated under the current protocol number. If the acceptance criterion for the neutralization confirmation control, initial population control or carrier population control (post-testing) is not met and the test substance fails to meet the test substance performance criteria, no repeat testing is necessary. Any positive test carriers confirmed as a contaminant will be reported. Any test carrier set that demonstrates a number of contaminated tubes that contributes to results that exceed the product performance/success criteria may be invalidated per Sponsor's request and may be re-tested.

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

METHOD FOR CONTROL OF BIAS: N/A

REPORT

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

PROTOCOL CHANGES

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

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

TEST SUBSTANCE RETENTION

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

RECORD RETENTION

Study Specific Documents

All the original raw data developed exclusively for this study shall be archived at Element Materials Technology Eagan following the record retention policy outlined in the internal SOP ALS-0032. These original data include, but are not limited to, the following:

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

Facility Specific Documents

The following records shall also be archived at Element Materials Technology Eagan. These documents include, but are not limited to, the following:

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

REFERENCES

1. Association of Official Analytical Chemists (AOAC) Official Method 961.02, Germicidal Spray Products as Disinfectants. In Official Methods of Analysis of the AOAC, 2012 Edition.
2. Association of Official Analytical Chemists (AOAC) Official Method 955.17, Fungicidal Activity of Disinfectants. In Official Methods of Analysis of the AOAC, 1955 Edition.
3. U.S. Environmental Protection Agency, Registration Division, Office of Pesticide Programs, Draft Protocol "Protocol for Sterilization of Porous and Non-Porous Surfaces within Sealed Enclosures using Vaporized Hydrogen Peroxide".
4. 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.
5. 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.

DATA ANALYSIS

Calculations

Determine the CFU/carrier in the Initial Population Control, Carrier Population Control (post-testing) and non-active treatment control using all average counts between 0-300 CFU as follows:

$$\text{CFU/carrier} = \frac{(\text{avg. CFU for } 10^{-4}) + (\text{avg. CFU for } 10^{-5}) + (\text{avg. CFU for } 10^{-6}) \times (\text{Volume of neutralizer})}{[10^{-4} + 10^{-5} + 10^{-6}] \times (\text{Volume plated}) \times (\# \text{ of carriers per set})}$$

where 10^{-4} , 10^{-5} , and 10^{-6} are example dilutions that may be used

Statistical Analysis

None Used

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STUDY INFORMATION

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

Fogging/Misting/Vaporizing Device Identity (exactly as it should appear on final report):

The VaPure Device

Test Substance (Name & Batch Numbers) exactly as it should appear on final report:

Test Substance Name	Lot/Batch Number
VigorOx Liquid Sanitizer and Disinfectant	03173-4
VigorOx Liquid Sanitizer and Disinfectant	03173-5

Two batches of test substance are required for registration purposes. The requirement to test at the lower certified limit (LCL) for registration has not yet been defined for this organism by the U.S. EPA; however, testing at the LCL is recommended.

Test Substance Description:

- ☐ Quaternary ammonia ☒ Peracetic acid ☐ Sodium hypochlorite
☐ Iodophor ☒ Peroxide ☐ Other _____

Approximate Test Substance Active Concentration (upon submission to Element Materials Technology Eagan):

Hydrogen Peroxide- 21.7% , Peroxyacetic acid – 5.1%

(This value is used for neutralization planning only. This value is not intended to represent characterization values.)

Non-Active Control Substance (Name & Batch Numbers) exactly as it should appear on final report:

OR

☒ Use Sterile Deionized Water

Neutralization/Subculture Broth:

- ☐ _____
☒ Element Materials Technology Eagan Discretion. By checking, the Sponsor authorizes Element Materials Technology Eagan, at their discretion, to perform neutralization confirmation assays at the Sponsor's expense prior to testing to determine the most appropriate neutralizer. (See Fee Schedule).

Storage Conditions:

- ☒ Room Temperature
☐ 2-8°C
☐ Other: _____

Hazards:

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

Product Preparation

☐ No dilution required, Use as received (RTU)

☒ Dilution(s) to be tested:

$\frac{19.2 \text{ oz/gallon}}{\text{(example: 1 oz/gallon)}}$ defined as $\frac{19.2 \text{ oz}}{\text{(amount of test substance)}} + \frac{108.8 \text{ oz}}{\text{(amount of diluent)}}$

- ☒ 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
☐ Other _____

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

Test Organism: ☒ *Aspergillus niger* (ATCC 6275)

Carrier Number: 22/cycle/organism

Organic Soil Load:

- ☒ Minimum 5% Organic Soil Load (Fetal Bovine Serum) will be included in the test organism suspension
☐ No Organic Soil Load Required

Template: 247-I Rev. 0038

— Proprietary Information —

1285 Corporate Center Drive, Suite 110 • Eagan, MN 55121 • 877.287.8378 • 651.379.5510 • <http://www.element.com>

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Estimated Cycle Parameters

Estimated Cycle Time from activation to completion: 30 min device run time + 45 min dwell time + room exhaust time

Expected Exposure time in the cycle: 30 min device run time + 45 min dwell time

Desired Temperature at the Start of Exposure: Ambient conditions (18-25°C)

NOTE: The HVAC system will be turned off during exposure to ensure the vents are sealed. Element Materials Technology Eagan can only target the desired temperature at the start of each cycle.

Instructions for Filling the Device with the substance

To be performed by sponsor

Equipment Operational/Activation Instructions

To be performed by sponsor

☒ Check if applicable: The equipment will be operated by the Sponsor or Sponsor Representative on the day of testing. (This will be noted in the GLP compliance statement of the GLP final report.)

Additional Testing Room Setup Requirements (check all that apply):

☐ Not applicable

☒ 1 (#) supplemental fan(s) shall be used to aid with test substance distribution.

☒ A humidifier should be placed in the room to target a minimum of 50 % relative humidity prior to the start of the cycle.

☐ A dehumidifier should be placed in the room to achieve ≤ _____ % relative humidity prior to the start of the cycle.

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Active Ingredient Monitoring During Test Cycles

The following Sponsor-provided chemical indicators will be used:

NA

OR

The following Sponsor-provided detection probe/device will be used:

NA

Instructions for use of the indicators or detection probe (include instructions for use and evaluation):

NA

Expected concentration of active to be achieved in the test cycle based on the indicator or probe:

*Read and Record using Element provided Gas monitors at end of device run time.
Per 8-15-24 Email, Tue 8-15-24*

Safe re-entry into the room

☒ The room exhaust will be turned on high (clearing about 1000 cubic feet per minute or 28 cubic meters per minute) for at least 8 more minutes prior to entry. This will clear the room at least 2 times.

The following Sponsor provided monitoring device will be used on the day of testing to ensure safe re-entry into the room: _____

The reading must be at or below a reading of ≤4 ppm for peroxyacetic acid and ≤ 1 ppm for Hydrogen Peroxide to enter the room.

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Carrier Placement

☐ Carriers will be placed as outlined in figure 1 within the protocol

OR

☒ The following modified locations will be made: See protocol attachment I.

Room Modifications (if applicable)

☐ The Element Materials Technology Eagan' testing room will be used for testing. The actual dimensions of the testing room enclosure are: 18'8.5" x 16'8" x 11'9" (length x width x height), totaling approximately 3663.7 feet³ or 104 m³.

OR

☒ The following modifications will be made to the room: See protocol attachment I.

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TEST SUBSTANCE SHIPMENT STATUS

(This section is for informational purposes only.)

- ☐ Test Substance is already present at Element Materials Technology Eagan.
☒ Test Substance has been or will be shipped to Element Materials Technology Eagan.
Date of expected receipt at Element Materials Technology Eagan: _____
☐ Test Substance to be hand-delivered (must arrive by noon at least one day prior to testing or other arrangements made with the Study director)

COMPLIANCE

Study to be performed under EPA Good Laboratory Practice regulations (40 CFR Part 160) and in accordance to standard operating procedures.

- ☒ Yes
☐ No (Non-GLP or Development Study)

TESTING FACILITY MANAGEMENT VERIFICATION OF 40 CFR PART 160 SUBPART B (160.31(D))

Identity, strength, purity, and uniformity, as applicable, of the test lots has been or will be completed prior to efficacy testing: ☒ Yes ☐ No* ☐ Not required, Non-GLP testing requested

If yes, testing was or will be performed following 40 CFR Part 160 GLP regulations: ☒ Yes ☐ No*

Optional information to complete as applicable:

- ☒ 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 under protocol or study #:

Stability testing of the formulation has been or will be completed prior to or concurrent with efficacy testing:

☒ Yes ☐ No* ☐ Not required, Non-GLP testing requested

If yes, testing was or will be performed following 40 CFR Part 160 GLP regulations: ☒ Yes ☐ No*

Optional information to complete as applicable:

- ☐ Testing has been or will be conducted under protocol or study #:

**If 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.*

PROTOCOL MODIFICATIONS

- ☐ Approved without modification
☒ Approved with additional modifications
See protocol attachment.

PROTOCOL ATTACHMENTS

Supplemental Information Form Attached - ☒ Yes ☐ No

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PROPRIETARY INFORMATION

THIS DOCUMENT IS THE PROPERTY OF AND CONTAINS PROPRIETARY INFORMATION OF ELEMENT MATERIALS TECHNOLOGY EAGAN. NEITHER THIS DOCUMENT, NOR INFORMATION CONTAINED HEREIN IS TO BE REPRODUCED OR DISCLOSED TO OTHERS, IN WHOLE OR IN PART, NOR USED FOR ANY PURPOSE OTHER THAN THE PERFORMANCE OF THIS WORK ON BEHALF OF THE SPONSOR, WITHOUT PRIOR WRITTEN PERMISSION OF ELEMENT MATERIALS TECHNOLOGY EAGAN.

APPROVAL SIGNATURES

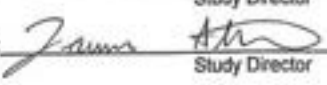
SPONSOR:

NAME: Mr. Brandon Adams TITLE: CEO
SIGNATURE:  DATE: 7/22/24
PHONE: 801 628 4667 EMAIL: brandon@puremaintenance.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

Element Materials Technology Eagan:

NAME: Tanner Strawn
Study Director
SIGNATURE:  DATE: 8-15-24
Study Director

Attachment I for PUR006071924.FRDT.1 *rev 3-15-24*



Fabric Preparation

Prepare a scouring solution by adding approximately 1.5 grams Na_2CO_3 and approximately 1.5 grams of Triton X-100 to approximately 3 L of deionized water (or equivalent dilution). The test fabric will be plain cotton weave containing approximately 80 x 80 threads/inch. Add approximately 300 grams of test fabric to each 3 L volume of scouring solution, or equivalent. Allow the solution to boil for approximately 60 minutes. Remove the fabric and rinse by placing first in boiling water for a minimum of five minutes and then placing in cold water for a minimum of five minutes. During the rinsing procedure, mix the fabric in the water in order to help remove the wetting agent. Allow the fabric to air dry under ambient conditions for at least 1 day ensuring that the material is completely dry. An alternate fabric type may be evaluated by Sponsor request.

Carriers

To represent porous surfaces and to ensure compatibility with the placement of the surfaces in the testing room, four inch by one inch fabric carriers will be wrapped around three inch by one inch clean glass microscope slides. Clean slides and fabric carriers will be steam sterilized or sterilized in a hot air oven at $\geq 180^\circ\text{C}$ for ≥ 2 hours. One prepared carrier will be transferred into a sterile Petri dish matted with 2 pieces of filter paper.

Contamination of Carriers

The carriers will each be inoculated with 10 μL culture, using a 4 mm loop or calibrated pipettor uniformly spreading the culture over an approximate 1 inch x 1 inch area on the end of the slide in a Petri dish. Following inoculation, the carriers will be dried for 30-40 minutes at $35\text{-}37^\circ\text{C}$.

Preparation of Enclosed Space

A visibly clean and sealed bioaerosol room of approximately $3,870\text{ ft}^3$ will be used for testing. The room will be appropriately sealed prior to testing to isolate the space in which the test substance is introduced. At a minimum, this will include an initial air purge to replace air within the room with HEPA filtered air. The room will be conditioned to target the requested temperature and relative humidity conditions.

Shelves with supports, laboratory carts or other suitable means of supporting the test carriers will be positioned within the enclosure.

For room sizes $\leq 100\text{ m}^3$, a minimum of 20 carriers must be evaluated per test organism, per test cycle. Triplicate carriers at a minimum of twenty designated locations required to substantiate efficacy claims.

The testing room enclosure is approximately $21.5' \times 15' \times 12'$ (length x width x height), totaling approximately $3,870\text{ ft}^3$ (110 m^3), therefore a minimum of 22 carriers would be required for testing, per cycle. An additional location has been added to achieve the minimum required number of test carriers. See Figure 1 below on the following page. The test criteria for passing the test remains the same for 22 carriers.

The actual volume of the testing enclosure will be reported.

Attachment I for PUR006071924.FRDT.1

TAA₃-15-24

Carrier Placement in the Enclosed Space



For all supplemental organisms including fungi, for each batch of test substance, a single carrier will be evaluated in each of twenty designated locations, described in figure 1, yielding 22 test carriers per test organism. These locations are designed to evaluate the following areas of the room:

1. All corners
2. Central locations on all wall faces
3. Central locations on the floor
4. Multiple locations and heights
5. Underneath horizontal surfaces

Each carrier will be oriented horizontally with the inoculum facing up. This will be accomplished by placing the Petri dish containing the carrier in the enclosure with the dish lid removed.

Figure 1: Sample Diagram of Carrier Placement in a Room Enclosure

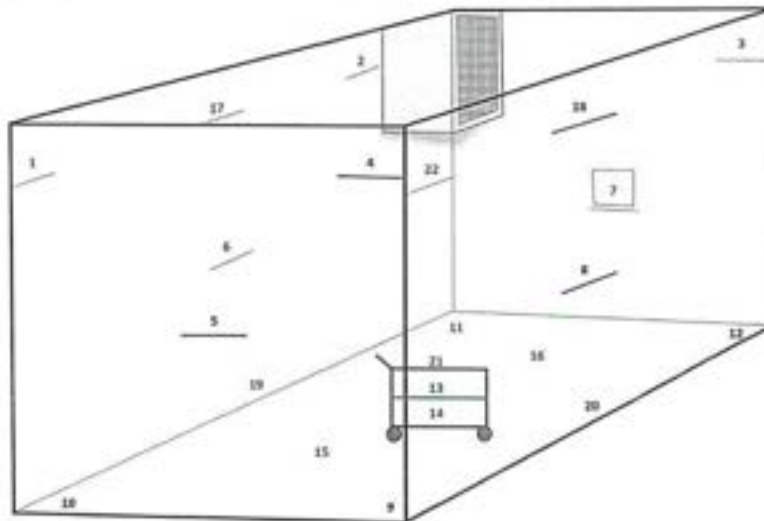


Figure 1: Illustration of a testing room, not necessarily to scale. Shelves 1-4 represent upper corners of the room. Shelves 5-8 represent central locations on all wall faces (Shelf 7 is directly under a viewing window). Spaces 9-12 represent the lower corners of the room. Spaces 13 and 14 on a 3-tier laboratory cart represent spaces underneath horizontal surfaces. Spaces 15 and 16 represent central locations very near the floor. Spaces 17 and 18 represent upper central wall faces, spaces 19 and 20 represent lower central wall faces and space 21 represents a central location on the top of the laboratory cart. Space 22 represents an upper corner beneath the testing room exhaust return. Location 22 was added to ensure 22 carriers could be evaluated in testing.

Attachment I for PUR006071924.FRDT.1

Aug. 15-24

 element

Exposure Conditions

Once the required environmental conditions have been achieved and the carriers have been positioned, the HVAC system and exhaust fan will be turned off to ensure the vents dampers are closed. The exhaust fan will be confirmed to be off.

The test device will be activated per Sponsor direction and the technician will immediately exit the room.

The cycle will be allowed to continue until completion. The total time from activation to the completion of cycle will be documented.

The Sponsor will be responsible for ensuring there is a mechanism in place to confirm safe re-entry into the room. In addition, the room exhaust will be turned on high (clearing about 1000 cubic feet per minute or 28 cubic meters per minute) for at least 8 more minutes prior to entry. This will clear the room at least 2 times. The Sponsor may supply a room scrubber or other aeration device to aid in clearing the room to speed up re-entry. An internal purge cycle with up to 5 air changes per hour may also be used as deemed necessary by the Study Director.

Test Substance Performance Criteria

In order to be considered effective for fungicidal efficacy, the test substance must kill the test organism on all 22 inoculated carriers.

REFERENCES

1. Association of Official Analytical Chemists (AOAC) Official Method 955.17, Fungicidal Activity of Disinfectants. In Official Methods of Analysis of the AOAC, 1955 Edition.
2. U.S. Environmental Protection Agency, Registration Division, Office of Pesticide Programs, Draft Protocol "Protocol for Sterilization of Porous and Non-Porous Surfaces within Sealed Enclosures using Vaporized Hydrogen Peroxide".
3. U.S. Environmental Protection Agency, Registration Division, Office of Pesticide Programs, Draft Protocol "Protocol for Sterilization of Porous and Non-Porous Surfaces within Sealed Enclosures using Vaporized Hydrogen Peroxide".
4. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, OCSPP 810.2000: General Considerations for Testing Public Health Antimicrobial Pesticides – Guidance for Efficacy Testing, February 2018.
5. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, OCSPP 810.2200: Disinfectants for Use on Environmental Surfaces – Guidance for Efficacy Testing, February 2018.
6. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, Series 810 Guidelines FAQ, August 2019.
7. U.S. Environmental Protection Agency, Office of Pesticide Programs SOP Number: MB-30-03, Preparation of Hard Water and Other Diluents for Preparation of Antimicrobial Products, November 14, 2022.