



## **FINAL STUDY REPORT**

### **STUDY TITLE**

Standard Test Method for Efficacy of Sanitizers  
Recommended for Inanimate Non-Food Contact Surfaces

### **Test Organism(s):**

*Escherichia coli* O157:H7 (ATCC 35150)

### **PRODUCT IDENTITY**

Peraguard  
Lot JDNB6-12-1 and Lot JDNB6-12-2

### **TEST GUIDELINE**

OCSPP 810.2300

### **PROTOCOL NUMBER**

ENV003110719.NFS.2

### **AUTHOR**

Kristin Hunt, B.S.  
Study Director

### **STUDY COMPLETION DATE**

December 11, 2019

### **PERFORMING LABORATORY**

Analytical Lab Group-Midwest  
1285 Corporate Center Drive, Suite 110  
Eagan, MN 55121

### **SPONSOR**

Enviro Tech Chemical Services  
500 Winmoore Way  
Modesto, CA 95358

### **PROJECT NUMBER**

A28828



**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: Enviro Tech Chemical Services

Company Agent:

Tina Rodrigues

Regulatory Affairs

Title

Tina Rodrigues

Signature

Date: 12/12/19



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### 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: Jane Rodgus Date: 12/12/19

Sponsor: Jane Rodgus / EnviroTechChemical Date: 12/12/19

Study Director: Kristin Hunt Date: 12/11/19  
Kristin Hunt, B.S.



### QUALITY ASSURANCE UNIT SUMMARY

Study: Standard Test Method for Efficacy of Sanitizers Recommended for Inanimate Non-Food Contact Surfaces

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

Phase Inspected	Date of Phase Inspection	Date Reported to Study Director	Date Reported to Management
Critical Phase Audit: Contamination of Carriers	November 21, 2019	November 21, 2019	November 25, 2019
Final Report	December 11, 2019	December 11, 2019	December 11, 2019

Quality Assurance Specialist: \_\_\_\_\_

*Jenaj Haebel*

Date: 12/11/19





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## STUDY PERSONNEL

STUDY DIRECTOR: Kristin Hunt, B.S.

Professional personnel involved:

Shanen Conway, B.S.	- Manager, Study Director Operations
Amy Backler, M.S.	- Manager, Core Services Laboratory Operations
Tanner Barnharst, M.S.	- Microbiologist
James Walrath, B.S.	- Microbiologist
Adam Meyer, B.S.	- Microbiologist
Miranda Quist, B.S.	- Microbiologist
Kaitlyn Baldrige, B.A.	- Associate Microbiologist
Ashley Millerbernd, B.A.	- Associate Microbiologist



## STUDY REPORT

### GENERAL STUDY INFORMATION

**Study Title:** Standard Test Method for Efficacy of Sanitizers Recommended for Inanimate Non-Food Contact Surfaces

**Project Number:** A28828

**Protocol Number:** ENV003110719.NFS.2

**Sponsor:** Enviro Tech Chemical Services  
500 Winmoore Way  
Modesto, CA 95358

**Test Facility:** Analytical Lab Group-Midwest  
1285 Corporate Center Drive, Suite 110  
Eagan, MN 55121

### TEST SUBSTANCE IDENTITY

**Test Substance Name:** Peraguard

**Lot/Lot(s):** Lot JDNB6-12-1 and Lot JDNB6-12-2

**Manufacture Date(s):** November 7, 2019 (Lot JDNB6-12-1)  
November 7, 2019 (Lot JDNB6-12-2)

### 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 Attachments I-II.

### STUDY DATES

**Date Sample Received:** November 14, 2019

**Study Initiation Date:** November 18, 2019

**Experimental Start Date:** November 21, 2019 (Start time: 9:00 am)

**Experimental End Date:** November 25, 2019 (End time: 11:00 am)

**Study Completion Date:** December 11, 2019

### OBJECTIVE

The objective of this study was to determine the antimicrobial efficacy of sanitizers on hard, inanimate, non-porous, non-food contact surfaces. This method is in compliance with the requirements of the U.S. Environmental Protection Agency (EPA).





## **SUMMARY OF RESULTS**

Test Substance: Peraguard (Lot JDNB6-12-1 and Lot JDNB6-12-2)  
Dilution: g/ Liter, defined as 35.6 grams of test substance + 1 Liter 400 ppm AOAC Synthetic Hard Water  
Test Organism(s): *Escherichia coli* O157:H7 (ATCC 35150)  
Exposure Time: 5 minutes  
Exposure Temperature: Room temperature (19°C)  
Organic Soil Load: No organic soil load required  
Efficacy Result: Peraguard demonstrated efficacy of two out of two lots against *Escherichia coli* O157:H7, and therefore, meets the performance requirements set forth by the U.S. EPA following a 5 minute exposure time at room temperature (19°C).

## **STUDY MATERIALS**

### **Test System/Growth Media**

Test Organism	Designation #	Growth Medium	Incubation Parameters
<i>Escherichia coli</i> O157:H7	35150	Synthetic Broth	35-37°C, aerobic

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

### **Recovery Media**

Neutralizer: D/E Neutralizing Broth + 0.01% Catalase

Agar Plate Medium: Tryptic Soy Agar with 5% Sheep's Blood (BAP)

### **Reagents**

#### **Hard Water Description:**

The Sponsor specified 400 ppm AOAC synthetic Hard Water was made using 12.0 mL of AOAC Solution I and 12.0 mL of AOAC Solution II. The total volume of the solution was brought to approximately 3 L using sterile deionized water. The synthetic hard water was prepared, titrated, and used for testing on the day of preparation. The actual titration result was 397 ppm.

### **Carriers**

Glass 1" x 1" carriers were dipped in 95% alcohol, rinsed with deionized water, and air dried before sterilization. The carriers were placed into a vessel and sterilized in a hot air oven for ≥2 hours at ≥180°C. After sterilization, each carrier was placed into a sterile Petri dish.





## **TEST METHOD**

### **Preparation of Test Substance**

An equivalent dilution of g/Liter, defined as 35.6 grams of test substance + 1 Liter diluent, was prepared using 3.56 grams of the test substance and 100.0 mL of 400 ppm AOAC Synthetic Hard Water. The prepared test substance was mixed for 5 minutes prior to use and was homogenous as determined by visual observation. The prepared test substance was used within 30 minutes of preparation.

### **Preparation of Test Organism**

From a stock slant no more than 5 transfers from original stock and  $\leq 1$  month old, an initial tube (10 mL) of culture broth was inoculated. This culture was termed the "initial broth suspension." From this initial broth suspension, a minimum of three daily transfers using 1 loopful (10  $\mu$ L) of culture into 10 mL of culture media was performed on consecutive days prior to use as an inoculum. Each daily transfer was incubated at 35-37°C (36.0°C) for 24 $\pm$ 2 hours using the appropriate growth medium.

A 48-54 hour (48 hour) culture that was incubated at 35-37°C (36.0°C) was vortex-mixed and allowed to settle for  $\geq 15$  minutes. The upper 2/3rds of the culture was removed and transferred to a sterile vessel for use in testing. The culture was thoroughly mixed prior to use.

### **Contamination of Carriers**

Sterile carriers were inoculated with 0.02 mL (20.0  $\mu$ L) of culture using a calibrated pipettor spreading the inoculum to within approximately 3 mm of the edges of the carrier. The inoculated carriers were dried for 21 minutes at 35-37°C (36.2°C) and 48.2-50.1% relative humidity with the Petri dish lids slightly ajar and appeared visibly dry following drying. A constant humidity chamber was used in place of a desiccating chamber to ensure uniform humidification conditions and to overcome slow re-equilibration of a desiccator after opening.

### **Exposure Conditions**

Following the completion of drying, each of the five test carriers were transferred to individual sterile 2 oz. (60 mL) polypropylene jars using sterile forceps with the inoculum facing up. Using staggered intervals, 5.0 mL of prepared test substance was transferred to each jar. The liquid completely covered the carriers during exposure. The carriers were allowed to expose at room temperature (19°C) and 34% relative humidity for 5 minutes. Following exposure, 20 mL of neutralizer was transferred to the jars using identical staggered intervals. The jars were vortex-mixed for 10 seconds to suspend the surviving organisms.

### **Test System Recovery**

Within 30 minutes of neutralization, duplicate 1.00 mL and 0.100 mL aliquots of the neutralized solution ( $10^0$ ) were plated onto the recovery agar plate medium.



### **Incubation and Observation**

The subculture plates were incubated at 35-37°C (36.0°C) for 48±4 hours (47.25 hours). The subcultures were placed at 2-8°C for 2 days prior to examination. Following incubation and storage, the subcultures were visually enumerated.

### **STUDY CONTROLS**

#### **Carrier Population Control**

Three inoculated, dried control carriers were treated as in the test procedure utilizing sterile deionized water in place of test substance. The carriers were exposed for the shortest exposure time followed in the test procedure. Following exposure, the carriers were neutralized as in the test and mixed as in the test. Ten-fold serial dilutions were prepared and duplicate 0.100 mL aliquots of the 10<sup>-1</sup> through 10<sup>-4</sup> dilutions were plated onto an appropriate agar. The plates were incubated as in the test procedure and enumerated. The acceptance criterion for this control is a minimum geometric mean value of 2.5 x 10<sup>4</sup> CFU/carrier which is required to show a 99.9% reduction.

#### **Carrier Sterility Control**

Concurrent with testing, a representative, uninoculated carrier was added to the neutralizer. The vessel was mixed and 1.00 mL was plated onto appropriate agar and incubated. The acceptance criterion is a lack of growth following incubation.

#### **Neutralizer Sterility**

Concurrent with testing, a 1.00 mL aliquot of neutralizer was plated onto appropriate agar and incubated. The acceptance criterion is a lack of growth following incubation.

#### **Culture Purity**

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

#### **Neutralization Confirmation Control**

In a manner consistent with the AOAC 960.09 method, the neutralization confirmation control was performed concurrent with testing.

The prepared test culture was serially diluted to target 2x10<sup>4</sup> – 2x10<sup>5</sup> CFU/mL (to target a result of 10-100 CFU plated in each control run). Multiple organism dilutions were prepared.

#### **Test Culture Titer (TCT)**

A 0.100 mL aliquot of diluted test organism was added to 25.0 mL of sterile diluent and vortex mixed. The mixture was held for a minimum of 30 minutes and was then spread utilizing duplicate 0.100 mL and 1.00 mL aliquots using the same method used in the test. The acceptance criterion for this study control is growth.





#### **Neutralization Confirmation Control Treatment (NCT)**

A sterile carrier was immersed (one per test organism dilution to be used, per test substance to be evaluated) in 5.0 mL of test substance as in the test. The sterile carrier was allowed to expose for the exposure time and each carrier was neutralized with 20 mL of neutralizer. The jar was vortex-mixed for 10 seconds. Within 5 minutes, a 0.100 mL aliquot of diluted test organism was added to the neutralized contents and vortex mixed. The mixture was held for a minimum of 30 minutes and was then spread plated utilizing duplicate 0.100 mL and 1.00 mL aliquots using the same method used in the test. The acceptance criterion for this study control is growth within 1 log<sub>10</sub> of the test culture titer (TCT) for at least one of the aliquots plated.

#### **Neutralizer Toxicity Treatment (NTT)**

A 0.100 mL aliquot of diluted test organism was added to 25.0 mL of sterile neutralizer and was vortex mixed. The mixture was held for a minimum of 30 minutes and was then spread plated utilizing duplicate 0.100 mL and 1.00 mL aliquots using the same method used in the test. The acceptance criterion for this study control is growth within 1 log<sub>10</sub> of the test culture titer (TCT) for at least one of the aliquots plated.

#### **Inoculum Count**

The test organism was serially diluted and 0.100 mL aliquots of appropriate dilutions were plated in duplicate. The plates were incubated as in the test. This control is for informational purposes and therefore has no acceptance criterion.

### **STUDY ACCEPTANCE CRITERIA**

#### **Test Substance Performance Criteria**

The efficacy performance requirements for label claims state that the test substance must demonstrate a minimum 99.9% reduction of test survivors as compared to the population control to be considered an effective non-food contact sanitizer.

#### **Control Acceptance Criteria**

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

### **PROTOCOL CHANGES**

#### **Protocol Amendment(s):**

No protocol amendments were required for this study.

#### **Protocol Deviation(s):**

No protocol deviations occurred during this study.



## **DATA ANALYSIS**

### **Calculations**

$$\text{CFU/mL} = \frac{(\text{average CFU}) \times (\text{dilution factor})}{(\text{volume plated in mL})}$$

#### **Number of Organisms Surviving per Carrier**

$$\text{CFU/carrier} = \frac{(\text{average CFU}) \times (\text{dilution factor}) \times (\text{volume neutralized solution in mL})}{(\text{volume plated or filtered in mL})}$$

#### **Geometric Mean of Number of Organisms Surviving on Test or Control Carriers**

$$\text{Geometric Mean} = \text{Antilog of } \frac{\text{Log}_{10}X_1 + \text{Log}_{10}X_2 + \text{Log}_{10}X_N}{N}$$

where:

X equals CFU/carrier

N equals number of carriers

#### **Percent Reduction**

$$\% \text{ reduction} = [(a - b) / a] \times 100$$

where:

a = geometric mean of the number of organisms surviving on the population control carriers.

b = geometric mean of the number of organisms surviving on the test carriers.

**Recovery Log<sub>10</sub> Difference** = Log<sub>10</sub> (Average CFU in TCT) – Log<sub>10</sub> (Average CFU in NCT or NTT)  
Used for the neutralization confirmation control

### **Statistical Methods**

None used.





## **STUDY RETENTION**

### **Record Retention**

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

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

### **Test Substance Retention**

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

## **REFERENCES**

1. 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.
2. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, OCSPP 810.2300: Sanitizers for Use on Hard Surfaces- Efficacy Data Recommendations, September 4, 2012.
3. American Society for Testing and Materials (ASTM). Standard Test Method for Efficacy of Sanitizers Recommended for Inanimate Non-Food Contact Surfaces, E1153-14.
4. Association of Official Analytical Chemists (AOAC) Official Method 960.09, Germicidal and Detergent Sanitizing Action of Disinfectants Method. In Official Methods of Analysis of the AOAC, 2013 Edition.
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-30-02, Preparation of Hard Water and Other Diluents for Preparation of Antimicrobial Products, August 2019.
7. OECD Environment, Health and Safety Publications, Series on Testing Assessment No. 187 and Series on Biocides No. 6, Guidance Document on Quantitative Methods for Evaluating the Activity of Microbicides used on Hard Non-Porous Surfaces, June 21, 2013.



## **RESULTS**

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

All data measurements/controls including the culture purity, neutralizer sterility, carrier sterility, neutralization confirmation and carrier population controls were within acceptance criteria.

### **For Test Results, see Tables 5-6.**

## **ANALYSIS**

Peraguard (Lot JDNB6-12-1 and Lot JDNB6-12-2), diluted g/Liter, defined as 35.6 grams of test substance + 1 Liter 400 ppm AOAC Synthetic Hard Water, demonstrated a >99.99% reduction and a >99.99% reduction, respectively, of *Escherichia coli* O157:H7 (ATCC 35150) following a 5 minute exposure time when tested at room temperature (19°C).

## **STUDY CONCLUSION**

**Under the conditions of this investigation, Peraguard, diluted g/Liter, defined as 35.6 grams of test substance + 1 Liter 400 ppm AOAC Synthetic Hard Water, demonstrated efficacy against *Escherichia coli* O157:H7 as required by the U.S. EPA following a 5 minute exposure time at room temperature (19°C).**

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:

Type of Control	Results
	<i>Escherichia coli</i> O157:H7 (ATCC 35150)
Purity	Pure
Neutralizer Sterility	No Growth
Carrier Sterility	No Growth

**TABLE 2: INOCULUM CONTROL RESULTS**

Test Organism: <i>Escherichia coli</i> O157:H7 (ATCC 35150)			
Volume Plated	Dilution Factor		CFU/mL
	10 <sup>-6</sup>	10 <sup>-7</sup>	
0.100 mL	90, 93	21, 24	9.2 x 10 <sup>8</sup>

CFU = Colony Forming Units



**TABLE 3: NEUTRALIZATION CONFIRMATION CONTROL RESULTS**

Control Identity or Test Substance Identity	Dilution	Volume Plated (mL)	Survivors (CFU)	Test Culture Titer (TCT)	Log <sub>10</sub> Difference (Volume used)	Pass/Fail (± 1 Log <sub>10</sub> )
Neutralizer Toxicity Treatment (NTT)	10 <sup>-3</sup>	1.00	T, T	T, T	0.00 (1.00 mL)	Pass
	10 <sup>-4</sup>		T, T	T, T		
	10 <sup>-5</sup>		44, 37	41, 40		
	10 <sup>-3</sup>	0.100	T, T	T, T		
	10 <sup>-4</sup>		30, 32	33, 33		
	10 <sup>-5</sup>		3, 4	4, 7		
Peraguard Lot JDNB6-12-1 for NCT	10 <sup>-3</sup>	1.00	T, T	T, T	-0.04 (1.00 mL)	Pass
	10 <sup>-4</sup>		T, T	T, T		
	10 <sup>-5</sup>		43, 47	41, 40		
	10 <sup>-3</sup>	0.100	T, T	T, T		
	10 <sup>-4</sup>		31, 34	33, 33		
	10 <sup>-5</sup>		1, 3	4, 7		
Peraguard Lot JDNB6-12-2 for NCT	10 <sup>-3</sup>	1.00	T, T	T, T	0.02 (1.00 mL)	Pass
	10 <sup>-4</sup>		T, T	T, T		
	10 <sup>-5</sup>		35, 43	41, 40		
	10 <sup>-3</sup>	0.100	T, T	T, T		
	10 <sup>-4</sup>		30, 36	33, 33		
	10 <sup>-5</sup>		5, 3	4, 7		

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





**TABLE 4: CARRIER POPULATION CONTROL RESULTS**

Test Organism: <i>Escherichia coli</i> O157:H7 (ATCC 35150)							
Volume Plated: 0.100 mL							
Carrier #	Dilution Factor				CFU/ carrier	Log <sub>10</sub>	Geometric Mean (Average Log <sub>10</sub> )
	10 <sup>-1</sup>	10 <sup>-2</sup>	10 <sup>-3</sup>	10 <sup>-4</sup>			
1	T, T	34, 38	4, 6	1, 0	9.0 x 10 <sup>5</sup>	5.95	8.32 x 10 <sup>5</sup> (5.92)
2	T, T	33, 32	2, 6	0, 0	8.3 x 10 <sup>5</sup>	5.92	
3	T, T	30, 31	3, 3	0, 1	7.8 x 10 <sup>5</sup>	5.89	

CFU = Colony Forming Units  
 T = Too Numerous To Count (>300 colonies)

**TABLE 5: TEST CARRIER DATA**

Test Organism: <i>Escherichia coli</i> O157:H7 (ATCC 35150)				
Test Substance	Sample Dilution	Carrier #	Survivors at the 10 <sup>0</sup> dilution	
			1.00 mL	0.100 mL
Peraguard Lot JDNB6-12-1	g/Liter, defined as 35.6 grams of test substance + 1 Liter diluent	1	0, 0	0, 0
		2	0, 0	0, 0
		3	0, 0	0, 0
		4	0, 0	0, 0
		5	0, 0	0, 0
Peraguard Lot JDNB6-12-2	g/Liter, defined as 35.6 grams of test substance + 1 Liter diluent	1	0, 0	0, 0
		2	0, 0	0, 0
		3	0, 0	0, 0
		4	0, 0	0, 0
		5	0, 0	0, 0



**TABLE 6: TEST RESULTS**

<b>Test Organism: <i>Escherichia coli</i> O157:H7 (ATCC 35150)</b>						
<b>Test Substance</b>	<b>Carrier #</b>	<b>CFU/Carrier</b>	<b>Log<sub>10</sub></b>	<b>Average Log<sub>10</sub></b>	<b>Geometric Mean</b>	<b>Percent Reduction</b>
Peraguard Lot JDNB6-12-1	1	<2.5 x 10 <sup>1</sup>	<1.40	<1.40	<2.51 x 10 <sup>1</sup>	>99.99%
	2	<2.5 x 10 <sup>1</sup>	<1.40			
	3	<2.5 x 10 <sup>1</sup>	<1.40			
	4	<2.5 x 10 <sup>1</sup>	<1.40			
	5	<2.5 x 10 <sup>1</sup>	<1.40			
Peraguard Lot JDNB6-12-2	1	<2.5 x 10 <sup>1</sup>	<1.40	<1.40	<2.51 x 10 <sup>1</sup>	>99.99%
	2	<2.5 x 10 <sup>1</sup>	<1.40			
	3	<2.5 x 10 <sup>1</sup>	<1.40			
	4	<2.5 x 10 <sup>1</sup>	<1.40			
	5	<2.5 x 10 <sup>1</sup>	<1.40			

CFU = Colony Forming Units

A value of <1 was used in place of zero for calculation purposes.



**ATTACHMENT I: TEST SUBSTANCE CERTIFICATE OF ANALYSIS –  
LOT JDNB6-12-1**



ENVIRO TECH CHEMICAL SERVICES  
500 WINMOORE WAY  
MODESTO, CA 95358  
(209) 581-9576  
(209) 581-9653 FAX

**CERTIFICATE OF ANALYSIS      Peraguard**  
Peroxyacetic Acid and Hydrogen Peroxide

Prepared for: Analytical Lab Group

Product: Peraguard

Production Date: 11/7/19

Analysis Date: 11/8/19

Lot Number: JDNB6-12-1

Product diluted according to labeling:

Method: Ceric (4) sulfate/sodium thiosulfate

Results: (a) 5040 ppm H<sub>2</sub>O<sub>2</sub>

(b) 738 ppm PAA

Product neat:

		Target	min.	max.
Results:	(a) x 26.7/10,000 <u>3.5</u> % H <sub>2</sub> O <sub>2</sub>	14.3	13.6	15.0
	(b) x 26.7/10,000 <u>2.0</u> % PAA	2.1	2.0	2.2

Notes: Label concentration will be 37.5g / liter of water.  
For this lot, in order to achieve the lower certified limits, 35.6g  
of this product was diluted in 1 liter of water.

Mohet  
Technician

11/8/19  
Date

EXACT COPY  
INITIALS MM DATE 12-11-19



ATTACHMENT II: TEST SUBSTANCE CERTIFICATE OF ANALYSIS –  
LOT JDNB6-12-2



ENVIRO TECH CHEMICAL SERVICES  
500 WINMOORE WAY  
MODESTO, CA 95358  
(209) 581-9576  
(209) 581-9653 FAX

**CERTIFICATE OF ANALYSIS**      **Peraguard**  
Peroxyacetic Acid and Hydrogen Peroxide

Prepared for: Analytical Lab Group

Product: Peraguard

Production Date: 11/7/19

Analysis Date: 11/18/19

Lot Number: JDNB6-12-2

Product diluted according to labeling:

Method: Ceric (4) sulfate/sodium thiosulfate

Results: (a) 5052 ppm H<sub>2</sub>O<sub>2</sub>

(b) 745 ppm PAA

Product neat:

		Target	min.	max.
Results:	(a) x 26.7/10,000 <u>13.6</u> % H <sub>2</sub> O <sub>2</sub>	14.3	13.6	15.0
	(b) x 26.7/10,000 <u>2.0</u> % PAA	2.1	2.0	2.2

Notes: Label concentration will be 37.5g/Liter of water. For this lot, in order to achieve the lower certified limit, 35.6g of this product was diluted in 2 Liter of water.

Makoto  
Technician

11/18/19  
Date

EXACT COPY  
INITIALS MAK DATE 12-11-19





(For Laboratory Use Only)
Analytical Lab Group-Midwest Project # <u>A28828</u>
Test Substance Tracking <u>JS111419 ENV003</u>

KJP 11-19-19  
SKW  
11/15/19



**PROTOCOL**

**Standard Test Method for Efficacy of Sanitizers Recommended for Inanimate Non-Food Contact Surfaces**

**Test Organism(s):**

*Escherichia coli* O157:H7 (ATCC 35150)

**PROTOCOL NUMBER**

ENV003110719.NFS.2

**SPONSOR**

Enviro Tech Chemical Services  
500 Winmoore Way  
Modesto, CA 95358

**PERFORMING LABORATORY**

Analytical Lab Group-Midwest  
1285 Corporate Center Drive, Suite 110  
Eagan, MN 55121

**DATE**

November 7, 2019

EXACT COPY  
INITIALS SKW DATE 11-11-19



## Standard Test Method for Efficacy of Sanitizers Recommended for Inanimate Non-Food Contact Surfaces

### PURPOSE

The purpose of this study is to determine the antimicrobial efficacy of sanitizers on hard, inanimate, non-porous, non-food contact surfaces. This method is in compliance with the requirements of and may be submitted to, one or more of the following agencies as indicated by the Sponsor: U.S. Environmental Protection Agency (EPA) and Health Canada.

### TEST SUBSTANCE CHARACTERIZATION

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

### SCHEDULING AND DISCLAIMER OF WARRANTY

Experimental start dates are generally scheduled on a first-come/first-serve basis once Analytical Lab Group-Midwest receives the Sponsor approved/completed protocol, signed fee schedule and corresponding test substance(s). Based on all required materials being received at this time, the proposed experimental start date is November 25, 2019. Verbal results may be given upon completion of the study with a written report to follow on the proposed completion date of December 23, 2019. To expedite scheduling, please be sure all required paperwork and test substance documentation is complete/accurate upon arrival at Analytical Lab Group-Midwest.

If a test must be repeated, or a portion of it, due to failure by Analytical Lab Group-Midwest 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 Analytical Lab Group-Midwest nor any of its employees are to be used in advertising or other promotion without written consent from Analytical Lab Group-Midwest. 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 Analytical Lab Group-Midwest final report and notify Analytical Lab Group-Midwest of any perceived deficiencies in these areas before submission of the report to the regulatory agency. Analytical Lab Group-Midwest will make reasonable changes deemed necessary by the Sponsor, without altering the technical data.

### JUSTIFICATION FOR SELECTION OF THE TEST SYSTEM

The U.S. Environmental Protection Agency requires that a specific claim for a sanitizer be supported by appropriate scientific data demonstrating the efficacy of the sanitizer against the claimed organism. In addition, if applicable, Health Canada requires that the product be recognized as a disinfectant prior to accepting sanitizer claims. This is accomplished in the laboratory by treating the target 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 products intended for use on non-food contact surfaces, a carrier method is used in the generation of the supporting data. The test system to be used in this study follows the ASTM approved method for the evaluation of the antimicrobial efficacy of sanitizers on inanimate, nonporous, non-food contact surfaces.

### TEST PRINCIPLE

A film of organism cells dried on a surface of appropriate carriers is exposed to the test substance for a specified exposure time. After exposure, the carriers are neutralized and assayed for survivors. Appropriate sterility, culture purity, carrier population, neutralization confirmation and inoculum count controls are performed. The current revision of Standard Operating Procedure CGT-0032 reflects the methods which shall be used in this study.



## TEST METHOD

Table 1:

Test Organism	Designation #	Growth Medium	Incubation Parameters
<i>Escherichia coli</i> O157:H7	35150	Synthetic Broth	35-37°C, aerobic

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

**Subculture Agar:** Tryptic Soy Agar+5% Sheep's blood will be used in testing. The agar used in the test will be the same as that which is used in the control procedures which substantiates test organism recovery.

### Carriers

Glass 1" x 1" carriers shall be dipped in 95% alcohol, rinsed with deionized water, and air dried before sterilization. The carriers will be placed into a vessel and sterilized in hot air oven for  $\geq 2$  hours at  $\geq 180^\circ\text{C}$ . After sterilization, each carrier will be placed into a sterile Petri dish.

### Preparation of Test Organism

From a stock slant no more than 5 transfers from original stock and  $\leq 1$  month old, an initial tube (10 mL) of culture broth will be inoculated. This culture is termed the "initial broth suspension." From this initial broth suspension, at least three consecutive daily transfers using 1 loopful (10  $\mu\text{L}$ ) of culture into 10 mL of culture media will be performed prior to use as an inoculum. Incubate each daily transfer for  $24 \pm 2$  hours using the appropriate growth medium. The final test culture will be incubated for 48-54 hours.

A 48-54 hour culture will be vortex-mixed and allowed to settle for  $\geq 15$  minutes. The upper 2/3rds of the culture will be removed and transferred to a sterile vessel for use in testing. The culture may be adjusted by dilution in growth medium or by centrifuge concentration, if necessary. An organic soil load may be added to the test culture per Sponsor request. The test culture will be thoroughly mixed prior to use.

### Preparation of Test Substance

The test substance will be prepared according to the directions for intended use of the product. The test substance shall be used within three hours of preparation if additional preparation is required by Analytical Lab Group-Midwest.

### Contamination of Carriers

Inoculate each sterile carrier with 0.02 mL (20  $\mu\text{L}$ ) of culture using a calibrated pipettor spreading the inoculum to within approximately 3 mm of the edges of the carrier. Dry the inoculated carriers for 20-40 minutes until visibly dry. A drying humidity should be selected to encourage maximum survival of the test organism (targeting approximately 40% humidity, for example). The lids may be left slightly ajar or intact during drying if die-off is a concern. *The drying conditions for organisms not defined in the ASTM method have been modified to ensure adequate recovery of the test organism.* A constant humidity chamber will be used in place of a desiccating chamber to ensure uniform humidification conditions and to overcome slow re-equilibration of a desiccator after opening.

Drying Conditions: 35-37°C.





#### Exposure Conditions

Following the completion or drying, transfer each carrier to individual sterile 2 oz. (60 mL) polypropylene jars using sterile forceps with the inoculum facing up. Using staggered intervals, transfer 5.0 mL of prepared test substance to each jar. The liquid should completely cover the carrier during exposure. Continue treating the test carriers using staggered intervals. Allow the carriers to expose at the Sponsor specified exposure temperature for the Sponsor specified exposure time. Following exposure, transfer 20 mL of neutralizer to the jars using identical staggered intervals. Rotate the jar vigorously on an even plane for approximately 50 rotations to suspend the surviving organisms or vortex mix the jars for 10-15 seconds.

#### Test System Recovery

Within 30 minutes of neutralization, plate 1.0 mL and 0.1 mL aliquots of the neutralized subcultures ( $10^6$ ) in duplicate onto appropriate agar.

If neutralization of the test substance cannot be achieved chemically, filter-neutralization may be performed. Within 30 minutes of neutralization, transfer duplicate 1.0 mL and 0.1 mL of the neutralized solution, to individual filter units pre-wetted with 10 mL of sterile diluent. Evacuate the contents and rinse each filter with a minimum of 50 mL of sterile diluent. Transfer each filter to an appropriate agar using sterile forceps.

#### Incubation and Observation

All subcultures are incubated under the conditions listed in table 1 for  $48 \pm 4$  hours.

Following incubation, the subcultures will be visually enumerated. If necessary, the subcultures may be placed at  $2-8^\circ\text{C}$  for up to three days prior to examination.

Representative test plates showing growth may be subcultured, stained and/or biochemically assayed to confirm or rule out the presence of the test organism. If possible, subcultures containing 30-300 colonies will be used for calculations. When membrane filtration is used, the upper limit used for counting/calculations should be 200 CFU.

#### STUDY CONTROLS

##### Carrier Population Control

Inoculated, dried control carriers will be treated as in the test procedure utilizing sterile deionized water in place of test substance. If multiple exposure times were followed in testing, the carriers will be exposed for the shortest exposure time followed in the test procedure. Following exposure, the carriers will be neutralized as in the test. The carriers will be mixed as in the test. Ten-fold serial dilutions will be prepared and 0.1 mL aliquots of the  $10^{-1}$  to  $10^{-4}$  dilutions will be plated in duplicate. The plates will be incubated as in the test procedure and enumerated. The acceptance criterion for this study control is a minimum geometric mean value of  $2.5 \times 10^4$  CFU/carrier which is required to show a 99.9% reduction and has been modified for test organisms not defined in the ASTM method.

##### Carrier Sterility Control

Prior to testing, or concurrent with testing, a representative, uninoculated carrier will be added to the neutralizer. The vessel will be mixed and 1.0 mL will be plated onto appropriate agar and incubated. The acceptance criterion is a lack of growth following incubation.

##### Neutralizer Sterility

Prior to or concurrent with testing, a 1.0 mL aliquot of neutralizer will be plated onto appropriate agar and incubated. The acceptance criterion is a lack of growth following incubation.

##### Culture Purity

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



#### Organic Soil Sterility Control

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

#### Neutralization Confirmation Control

In a manner consistent with the AOAC 960.09 method, the following neutralization confirmation control will be performed prior to testing or concurrent with testing. To represent worst-case conditions, only the most concentrated test substance dilution and/or shortest exposure time needs to be utilized in this control when multiple test substance concentrations or multiple exposure times are being evaluated in the study.

Serially dilute the prepared test culture to target  $2 \times 10^4$  –  $2 \times 10^6$  CFU/mL (to target a result of 10-100 CFU plated in each control run). Multiple organism dilutions may be prepared. (Typically the  $10^{-3}$ ,  $10^{-4}$  or  $10^{-5}$  dilutions will provide a culture in range depending on expected titer. Alternate or partial dilutions may be used where appropriate.) If all the organism dilution(s) used in this control fail to provide adequate numbers which coincides in a failure to meet the acceptance criterion for this study control, the control may be repeated in its entirety.

#### Test Culture Titer (TCT)

Add 0.1 mL of diluted test organism to 25 mL of sterile diluent and vortex mix. Hold the mixture for a minimum of 30 minutes and spread plate or filter plate duplicate 1.0 mL and 0.1 mL aliquots using the same method used in the test. The acceptance criterion for this study control is growth. If the test culture titer fails to yield countable numbers or if the culture titer is too low resulting in failing results, the entire neutralization confirmation control may be repeated in its entirety, as necessary, to properly validate neutralization.

#### Neutralization Confirmation Control Treatment (NCT)

Immerse a sterile carrier (one per test organism dilution to be used, per test substance to be evaluated) in 5.0 mL of test substance as in the test. Expose for the exposure time and neutralize each carrier with 20 mL of neutralizer. Rotate the jar vigorously on an even plane for approximately 50 rotations or vortex mix the jars for 10-15 seconds. Within 5 minutes, add 0.1 mL of diluted test organism to the neutralized contents and vortex mix. Hold the mixture for a minimum of 30 minutes and spread plate or filter plate duplicate 1.0 mL and 0.1 mL aliquots using the same method used in the test. The acceptance criterion for this study control is growth within 1 log<sub>10</sub> of the test culture titer (TCT) for at least one of the aliquots plated.

#### Neutralizer Toxicity Treatment (NTT)

Add 0.1 mL of diluted test organism to 25 mL of sterile neutralizer and vortex mix. Hold the mixture for a minimum of 30 minutes and spread plate or filter plate duplicate 1.0 mL and 0.1 mL aliquots using the same method used in the test. The acceptance criterion for this study control is growth within 1 log<sub>10</sub> of the test culture titer (TCT) for at least one of the aliquots plated.

*Hold times after the addition of the test organism to the neutralization confirmation control vessels may be reduced if neutralization is a concern. Hold times followed should be as long or longer than the actual time required to plate the test carriers for a given test organism/test substance set.*

#### Inoculum Count

Serially dilute and plate the test organism in duplicate using 0.1 mL aliquots and appropriate dilutions and incubate as in the test. This control is for informational purposes and therefore has no acceptance criterion.





#### PROCEDURE FOR IDENTIFICATION OF THE TEST SYSTEM

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

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

**METHOD FOR CONTROL OF BIAS:** NA

#### STUDY ACCEPTANCE CRITERIA

##### **Test Substance Performance Criteria**

The efficacy performance requirements for label claims state that the test substance must demonstrate a minimum 99.9% reduction of test survivors as compared to the population control to be considered an effective non-food contact sanitizer.

##### **Control Acceptance Criteria**

The study controls must perform according to the criteria detailed in the study controls description section. If any control acceptance criteria are not met, the test may be repeated under the current protocol number.

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

#### REPORT

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

#### PROTOCOL CHANGES

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

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

#### TEST SUBSTANCE RETENTION

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





## RECORD RETENTION

### **Study Specific Documents**

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

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

### **Facility Specific Documents**

The following records shall also be archived at Analytical Lab Group-Midwest. 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. 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.
2. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, OCSPP 810.2300: Sanitizers for Use on Hard Surfaces- Efficacy Data Recommendations, September 4, 2012.
3. American Society for Testing and Materials (ASTM). Standard Test Method for Efficacy of Sanitizers Recommended for Inanimate Non-Food Contact Surfaces, E1153-14.
4. Association of Official Analytical Chemists (AOAC) Official Method 960.09, Germicidal and Detergent Sanitizing Action of Disinfectants Method. In Official Methods of Analysis of the AOAC, 2013 Edition.
5. Health Canada, January, 2014. Guidance Document – Safety and Efficacy Requirements for Hard Surface Disinfectant Drugs.
6. Health Canada, January, 2014. Guidance Document - Disinfectant Drugs.



## DATA ANALYSIS

### Calculations

$$\text{CFU/mL} = \frac{(\text{average CFU}) \times (\text{dilution factor})}{(\text{volume plated in mL})}$$

### Number of Organisms Surviving per Carrier

$$\text{CFU/carrier} = \frac{(\text{average CFU}) \times (\text{dilution factor}) \times (\text{volume neutralized solution in mL})}{(\text{volume plated or filtered in mL})}$$

### Geometric Mean of Number of Organisms Surviving on Test or Control Carriers

$$\text{Geometric Mean} = \text{Antilog of } \frac{\log_{10}X_1 + \log_{10}X_2 + \log_{10}X_N}{N}$$

Where: X equals CFU/carrier  
N equals number of carriers

### Percent Reduction

$$\% \text{ reduction} = [(a - b) / a] \times 100$$

where:

- a = geometric mean of the number of organisms surviving on the population control carriers.
- b = geometric mean of the number of organisms surviving on the test carriers.

**Recovery Log<sub>10</sub> Difference** = Log<sub>10</sub> (Average CFU in TCT) – Log<sub>10</sub> (Average CFU in NCT or NTT)  
Used for the neutralization confirmation control

**Statistical Methods**  
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.)

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

Test Substance Name	Lot/Batch Number
PERACETIC ACID	JUN16-13-1
PERACETIC ACID	JUN16-13-2

**Product Description:**

- Quaternary ammonia  
 Peroxide  
 Peracetic acid  
 Sodium hypochlorite  
 Iodophor  
 Other

Approximate Test Substance Active Concentration (upon submission to Analytical Lab Group-Midwest):  
13.5% H<sub>2</sub>O<sub>2</sub>, 2.0% DAA

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

Neutralization/Subculture Broth:

(NOTE: All broth must also serve as an appropriate growth medium for the test organism)

- Analytical Lab Group-Midwest Discretion. By checking, the Sponsor authorizes Analytical Lab Group-Midwest, 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:

1 liter defined as 35.6 g + 2 liter water  
(example: 1 oz/gallon) (amount of test substance) (amount of diluent)

Deionized Water (Filter or Autoclave Sterilized)

Tap Water (Filter or Autoclave Sterilized) - All tap water is softened; the water hardness for the batch of tap water used will be determined and reported.

AOAC Synthetic Hard Water: 400 PPM

Other

Let mix 5 minutes and use within 30 minutes

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

Test Organism(s):  *Escherichia coli* O157:H7 (ATCC 35150)

Carrier Number: 5 test carriers per batch and 3 population control carriers

Exposure Time: 5 Minutes

Exposure Temperature: Room temperature (18-25°C)

**Organic Soil Load:**

- Minimum 5% Organic Soil Load (Fetal Bovine Serum)  
 No Organic Soil Load Required  
 Other:





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TEST SUBSTANCE CURRENT STATUS

(This section is for informational purposes only.)

- Test Substance is already present at Analytical Lab Group-Midwest.  
 Test Substance has been or will be shipped to Analytical Lab Group-Midwest.  
Date of expected receipt at Analytical Lab Group-Midwest: ~ 11/22/19  
 Test Substance to be hand-delivered (must arrive by noon at least one day prior to testing or other arrangements made with the Study director).

COMPLIANCE

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

- Yes  
 No (Non-GLP or Development Study)

REGULATORY AGENCY(S) THAT MAY REVIEW DATA

- U.S. EPA  
 Health Canada

PROTOCOL MODIFICATIONS

- Approved without modification  
 Approved with modification

PROTOCOL ATTACHMENTS

Supplemental Information Form Attached -  Yes  No

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.



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**PROPRIETARY INFORMATION**

**THIS DOCUMENT IS THE PROPERTY OF AND CONTAINS PROPRIETARY INFORMATION OF ANALYTICAL LAB GROUP-MIDWEST. 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 ANALYTICAL LAB GROUP-MIDWEST.**

**APPROVAL SIGNATURES**

**SPONSOR:**

NAME: Ms. Tina Rodrigues TITLE: Regulatory Affairs & Lab Manager  
SIGNATURE: Tina Rodrigues DATE: 11/8/19  
PHONE: (209) 232 - 2208 EMAIL: trodrigues@envirotech.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

**Analytical Lab Group-Midwest:**

NAME: Kristin Hart  
Study Director  
SIGNATURE: Kristin Hart DATE: 11/8/19  
Study Director



NFS

For Analytical Lab Group-Midwest Protocol ENV003110719.NFS.2

Study Director Date/Initial: Yost 11-18-19

The following modifications will be made to align this protocol with the February 2018 version of the 810.2000 Product Performance Test Guidelines:

- a. The Product Performance Test Guidelines in the reference section, OCSPP 810.2000, will be updated to reflect the February 2018 version of the guidelines accordingly.
  - 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.
- b. Neutralization confirmation control will be confirmed concurrently with testing.
- c. The manufacture date of each product batch will be included in the report.

Lot/Batch Number	Manufacture Date
JDNBL-12-1	11/7/19
JDNBL-12-2	11/7/19
JDNBL-12-3	11/7/19 <sup>ⓐ</sup>

d. For any studies with presence of contamination in subculture media, a control failure, system failure, technician error, etc. the Repeat Testing Policy from the Series 810 Guidelines FAQ document will be followed.

e. Product Preparation:

- No dilution required, Use as received (RTU)
- \*Dilution(s) to be tested: g/liter defined as 35.0g + 1 liter water  
(example: 1 oz/gallon) (amount of test substance) (amount of diluent)
- OECD Hard Water: 375 ppm (338-394 ppm)
- Un-softened Tap Water: 200 ppm (180-210 ppm)
- AOAC Synthetic Hard Water: 400 ppm (360-420 ppm)
- Other \_\_\_\_\_

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

<sup>ⓐ</sup> Lot not applicable to protocol - 10/4 11-18-19





NFS

For Analytical Lab Group-Midwest Protocol ENV003110719.NFS.2

Study Director Date/Initial: \_\_\_\_\_ KCH 11-19-19

## f. Additional References (if applicable):

- i. U.S. Environmental Protection Agency, Office of Pesticide Programs SOP Number: MB-30-02, Preparation of Hard Water and Other Diluents for Preparation of Antimicrobial Products, August 2019.
- ii. OECD Environment, Health and Safety Publications, Series on Testing Assessment No. 187 and Series on Biocides No. 6, Guidance Document on Quantitative Methods for Evaluating the Activity of Microbicides used on Hard Non-Porous Surfaces, June 21, 2013.
- iii. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, Series 810 Guidelines FAQ, August 2019.

## g. OECD Hard Water Preparation (if applicable)

Sterile OECD hard water will be prepared by adding 6.0 mL of European hard water stock solution A to approximately 600 mL of sterile deionized water. Eight (8.0) mL of European hard water stock solution B will be added. The total volume will be adjusted to 1000 mL using deionized water. (Equivalent dilutions may be made). The pH of the hard water will be adjusted to  $7.0 \pm 0.2$ . The prepared water must be used within 24 hours of preparation. On the day of test, the water will be titrated and must demonstrate 338-394 ppm hardness. Appropriate solution adjustments may be made to target the final hardness concentration.