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G-202912
Project No.: GR2407
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Study Title

EPA Food Contact Sanitizer Test For Previously Cleaned Food-Contact Surfaces (AOAC Germicidal and Detergent Sanitizing Action of Disinfectants)

Product Identity

"Reflexx"

Data Requirement

EPA DIS/TSS-4 of January 30, 1979

Author

Daniel L. Prince, Ph.D.
President

Study Completion Date

6/30/2008

Testing Facility

Gibraltar Laboratories, Inc.
16 Montesano Road
Fairfield, NJ 07004

Laboratory Project Number (Study File)

GBL Study # GR 2407



Statement of No Data Confidentiality Claims

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA 10(d)(1)(A), (B) or (C).

Company ENVIRO TECH CHEMICAL SVCS, INC.

Company Agent MIKE HARVEY Date 8 JULY, 2008

Pres Michael Harvey
Title Signature

Note: Applicants for permanent or temporary tolerances should note that it is OPP Policy that no permanent or temporary tolerance petition or request for an emergency exemption, that incorporates an analytical method, can be approved unless the applicant waives all claims of confidentiality for the analytical method. These analytical methods are published in the FDA Analytical Methods Manual, and therefore cannot be claimed as confidential. OPP implements this policy by returning submitted analytical methods (for which confidentiality claims have been made) to the submitter, to obtain the confidentiality waiver before they can be processed.

*Gibraltar Laboratories, Inc. is not aware of what, if any, data will be classified as confidential. Accordingly, this page is left blank for the sponsor to complete.



GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study meets the requirements for 40 CFR Part 160 with the exception that the test agent stability information, synthesis, and purity analysis, composition and other characteristics of the test product remain with the sponsor.

Michael Harvey For:
SUBMITTER: Enviro Tech CS, Inc 7-8-08
Date

MICHAEL HARVEY
Study Submitter Name
Pres.
Study Submitter Title

SPONSOR: Enviro Tech Chemical Services, Inc.

Michael Harvey 7/8/08
Date
Michael Harvey
Study Contact Name

STUDY DIRECTOR: Jozef Mastej 7/1/08
Date
Jozef Mastej
Microbiology Manager



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QUALITY ASSURANCE STATEMENT

Study Title: EPA Food Contact Sanitizer Test For Previously Cleaned Food-Contact Surfaces (AOAC Germicidal and Detergent Sanitizing Action of Disinfectants)

Study Number: GR2407

In accordance with the Good Laboratory Practice Standards (EPA 40 CFR Part 160), quality assurance audits of this study were conducted and reported to management and the study director as listed below:

Audit Date	Phase Audited	Date Reported to Study Director	Date Reported to Management
06/20/2008	Procedure	06/20/2008	06/20/2008
06/20/2008	Facilities	06/20/2008	06/20/2008
06/26/2008	Data	06/26/2008	06/26/2008
06/27/2008	Report	06/27/2008	06/27/2008



Chuck Weibel
Quality Assurance Manager



Date



STUDY PERSONNEL

Testing Facility Management

Daniel L. Prince 7-1-08
Daniel L. Prince, Ph.D. Date
President

Study Director and
Supervisory Personnel

Jozef Mastej 7/1/08
Jozef Mastej Date
Microbiology Manager

Laboratory Personnel

M.A. Patel 7-1-08
Minal Patel Date
Microbiologist

Laboratory Personnel

Michael Pannullo 7-1-08
Michael Pannullo Date
Microbiologist



STUDY REPORT

STUDY TITLE: EPA Food Contact Sanitizer Test For Previously Cleaned Food-Contact Surfaces
(AOAC Germicidal and Detergent Sanitizing Action of Disinfectants)

SPONSOR: Enviro Tech Chemical Services, Inc.

500 Winmoore Way
Modesto, CA 95358
Attn: Michael Harvey
Tel #: 209/581-9576
Fax #: 209/581-9653
Sponsor #: (1124)
Purchase Order # 353584

TEST FACILITY: Gibraltar Laboratories, Inc.

16 Montesano Road
Fairfield, NJ 07004
Tel #: 973/227-6882
Fax #: 973/582-1565

TEST SUBSTANCE IDENTIFICATION

TEST SUBSTANCE NAME: "Reflexx"; an equilibrium mixture of Hydrogen Peroxide/Peroxyacetic Acid; Concentration on file at Enviro Tech Chemical Services.

LOT/BATCH NUMBER (S): Expiration date not known.

GBL # 207479/1 = Lot # 037-25-10032008; Manufacturing Date: March 10, 2008

GBL # 207479/2 = Lot # 037-25-21052008; Manufacturing Date: May 21, 2008

GBL # 207479/3 = Lot # 037-25-28052008; Manufacturing Date: May 28, 2008

DESCRIPTION OF TEST SUBSTANCE: Three clear glass bottles, each with a white plastic screw cap secured with black tape containing Reflexx. Expiration date is not known. Storage Conditions: The test materials were stored at ambient room temperature at the testing facility. Stability under storage conditions: Stability and purity are the responsibility of the sponsor.

CHEMICAL CHARACTERIZATION: The identity, solubility, stability, strength, purity, and chemical composition were not provided.



STUDY INITIATION DATE: 06/04/2008
EXPERIMENTAL START DATE: 06/20/2008
EXPERIMENTAL END DATE: 06/22/2008
STUDY COMPLETION DATE: 06/30/2008

STUDY OBJECTIVE: To determine whether or not "Reflexx", kills 99.999% of *Staphylococcus aureus* and *Escherichia coli* within 30 seconds in a suspension test.

TEST METHOD: AOAC 18th Edition Chapter 6.3.03; AOAC Official Method 960.09 "Germicidal and Detergent Sanitizing Action of Disinfectants"

TEST SYSTEM/STRAINS:

- *Staphylococcus aureus* (bacteria), GBL # 171952/8, ATCC # 6538
- *Escherichia coli* (bacteria), GBL # 171952/10, ATCC # 11229

Cultures received from American Type Culture Collection, Manassas, Virginia

The purity of the test system was confirmed by streaking onto selective agar and observing for characteristic morphological appearance (i.e., *S. aureus* = small yellow mannitol-fermenting colonies on Mannitol Salt Agar, *E.coli* = brick-red; may have surrounding zone of precipitated bile on MacConkey Agar).

STUDY MATERIALS

MEDIA AND REAGENTS

Nutrient Agar A Lot # F-104

Nutrient Agar B Lot # E-125

Phosphate Buffer Stock Solution, Lot # E-221

Phosphate Buffer Dilution Water, Lot # E-224

Tryptone Glucose Extract Agar Lot # E-352, F-29, 30

200 ppm AOAC Hard Water, Lot # C-1922

5% Sodium thiosulfate Lot # C1915

Catalase solution Lot # C-1913

Neutralizer Broth (Phosphate Buffer Dilution Water containing 57.2 units catalase / mL and 0.05% Sodium thiosulfate)

EQUIPMENT

Incubator 37 ± 1C

Water-bath 25 ± 0.2C

STUDY METHOD

PREPARATION OF TEST SUBSTANCE AND METHOD

One mL of the test substance was added to 767 mL of 200 ppm AOAC hard water (volume to volume). 99 mL of water to be used in the test, containing bactericide at the concentration to be tested, was measured into sterile, 250 mL wide-mouth Erlenmeyer flasks and placed in a constant temperature bath until it reached 25 ± 0.2C, for ≥20 min. Duplicate flasks were prepared for each germicide to be tested. A similar flask was also prepared containing 99 mL sterile phosphate buffer dilution H₂O, as "initial



numbers" control. One mL of culture suspension was added to each test flask as follows: The flask was whirled, stopping just before suspension was added, creating enough residual motion of liquid to prevent pooling of suspension at the point of contact with the test water. The suspension was added midway between center and edge of surface with tip of pipet slightly immersed in the test solution. Care was taken to avoid touching the pipet to the neck or side of flask during the addition. One mL portions of this exposed culture were added to neutralizer blank exactly 30 and 60 seconds after the addition of the suspension and mixed well immediately after transfer. For test samples, the following dilution procedure was followed: 1 mL-exposed culture was transferred into 9 mL neutralizer broth and vortexed to dislodge adhering organisms. 1 mL and 0.1 mL were plated in **quadruplicate** and poured with Tryptone Glucose Extract Agar (TGEA). Plates were incubated for 48 hours at $37 \pm 1C$. The colony forming units were counted using Quebec colony counter.

PREPARATION OF TEST SYSTEM/STRAINS

Staphylococcus aureus and *Escherichia coli* were prepared according to the AOAC 18th Edition, section 960.09D.

EXPOSURE CONDITIONS

Contact Time: 30 and 60 seconds

Organic Soil: none

Test Concentration: 1 ounce/ 6gallons (1:768)

Test Dilution: 1 mL test substance + 767 mL diluent

Diluent: sterile 200 ppm AOAC hard water

Test Temperature: $25 \pm 0.2C$

TEST SYSTEM RECOVERY

For incubation, the organisms will be incubated at $37 \pm 1C$ and not $35C$, as this is an acceptable temperature range for the mesophilic test system organisms for 48 ± 8 hours or longer according to the best judgment of the bacteriologist.

PROTOCOL CHANGES

PROTOCOL AMENDMENTS

None

PROTOCOL DEVIATIONS

None

CONTROLS

PREPARATION OF CONTROLS

Number Controls

The "number controls" were performed by adding 1 mL of the appropriate test system organism to **duplicate** 250 mL wide-mouth Erlenmeyer flasks containing 99 mL of the sterile phosphate buffer dilution water. The number of bacteria present in the Erlenmeyer flasks was determined after ≤ 30 seconds. Ten-fold serial dilutions were made into 9 mL neutralizer broth; 1 mL pour plates in



quadruplicate were performed using TGEA. Plates were incubated for 48 hours at $37 \pm 1C$. The colony forming units were counted using Quebec colony counter.

Neutralization Effectiveness Control

Neutralizer effectiveness was verified for each organism type and each lot germicide tested. 1.0 mL of the bactericide at the concentration to be tested was transferred into each sterile test tube containing 8 mL of neutralizer broth. 1 mL of 100 to 1000 cfu/mL of the test organism was inoculated into each test tube within 30 seconds and vortexed. 1 mL pour plates in **duplicate** were performed using TGEA. Plates were incubated for 48 hours at $37 \pm 1C$. The colony forming units were counted using Quebec colony counter.

Neutralization System Toxicity Control

Neutralizer system toxicity was verified for each organism type to assure that the neutralizing system is not toxic to the bacterial cells (If toxicity is present, the organism will not grow). 9 mL of neutralizer broth was tested into sterile test tube. 1 mL of 100 to 1000 cfu/mL of the test organism was inoculated into each test tube within 30 seconds and vortexed. 1 mL pour plates in **duplicate** were performed using TGEA. Plates were incubated for 48 hours at $37 \pm 1C$. The colony forming units were counted using Quebec colony counter.

Inoculum Counts

To verify the count of the inoculum is 10 to 100 cfu/mL, 1 mL of 100 to 1000 cfu/mL of the test organism was inoculated into each test tube containing 9 mL of Phosphate Buffer Dilution Water within 30 seconds and vortexed. 1 mL pour plates in **duplicate** were performed using TGEA. Plates were incubated for 48 hours at $37 \pm 1C$. The colony forming units were counted using Quebec colony counter.

Sterility Controls of Neutralizer Broth, AOAC Hard Water and Phosphate Buffer Dilution Water:

2 x 1.0 mL of Neutralizer broth, AOAC hard water was individually plated into sterile petri dishes and the plates were poured with TGEA. The plates were incubated at $37 \pm 1C$ for 48 ± 8 hours. The colony forming units were counted using Quebec colony counter.

STUDY ACCEPTANCE CRITERIA

STUDY REQUIREMENTS

- 1) Number Controls: counts between 75 and 125 x 10^6
Neutralizer Effectiveness: counts between 10 and 100 cfu/mL
Neutralizer Toxicity: counts between 10 and 100 cfu/mL
Inoculum Counts: counts between 10 and 100 cfu/mL
Sterility Controls: sterile
- 2) Performance criteria: the test substance must demonstrate a 99.999% kill within 30 seconds.



DATA ANALYSIS

CALCULATIONS

Basic arithmetic and transformation by Log₁₀

STATISTICAL ANALYSIS

None

STUDY RETENTION

Data Retention

The test findings reflected in this experiment will be kept on file for a period of at least five years in the Gibraltar Laboratories archives. Specific records to be maintained include a copy of this report, all raw data, sample information as provided by sponsor, and the findings of the QAU.

Specimen Retention

After all studies are complete the remaining test material, if any, will be discarded or destroyed in accordance with GBL policy and State and Federal regulations.

STUDY RESULTS

Control and Neutralization Results (Tables 5 and 6): Number controls, neutralizer effectiveness, neutralizer toxicity, inoculum counts and sterility control requirements were met.

Study Results (Table 1-4):

- Table 1-2 The test substance inactivated >99.999% of the *Staphylococcus aureus* within 30 seconds at 1ounce/6gallons (1:768) dilution at 25C.
- Table 3-4 The test substance inactivated >99.999% of the *Escherichia coli* within 30 seconds at 1ounce/6gallons (1:768) dilution at 25C.

STUDY CONCLUSION

Reflexx, Lot # 037-25-10032008, Lot # 037-25-21052008 and Lot # 037-25-28052008 prepared in 200 ppm AOAC hard water inactivated > 99.999% of *Escherichia coli* and *Staphylococcus aureus* when tested at 1ounce / 6gallons (1:768) dilution at 25C within 30 seconds contact time.

REPORT SUBMITTED BY:


Study Director
Jozef Mastej

7/1/08
Study Completion Date



Table 1: Raw Data Results for *Staphylococcus aureus*

Test Substance	Test Dilution (v/v)	Exposure Time	Flask	Plate Counts (CFU/plate)			
				Number Surviving		Number Controls (10 ⁶) (Microbes Initially Present)	
				10 ⁻¹	10 ⁻²	Flask A	Flask B
"Reflexx" Lot # 037-25-10032008	1 ounce/6gallons (1:768)	30 seconds	Flask A	76, 81, 61, 69	6, 7, 4, 7		
			Flask B	71, 69, 65, 56	8, 9, 7, 5		
		60 seconds	Flask A	0,0,0,0	0,0,0,0		
			Flask B	0,0,0,0	0,0,0,0		
"Reflexx" Lot # 037-25-21052008	1 ounce/6gallons (1:768)	30 seconds	Flask A	66, 71, 59, 81	8, 9, 6, 10		
			Flask B	61, 62, 74, 70	5, 9, 7, 6		
		60 seconds	Flask A	0,0,0,0	0,0,0,0		
			Flask B	0,0,0,0	0,0,0,0		
"Reflexx" Lot # 037-25-28052008	1 ounce/6gallons (1:768)	30 seconds	Flask A	56, 60, 71, 64	7, 8, 4, 5		
			Flask B	72, 74, 68, 79	9, 7, 6, 6		
		60 seconds	Flask A	0,0,0,0	0,0,0,0		
			Flask B	0,0,0,0	0,0,0,0		
						Avg. of Flask A and B 7.6 x 10 ⁷ = 7.88 Log	

Table 2: Calculated Results for *Staphylococcus aureus* (cfu/mL) by Lot, Exposure, and corresponding Percent and Log₁₀ Reduction

Test Substance / Lot #	Exposure Time	Test Dilution (v/v)	Average Number Surviving (cfu/mL)	Microbes Initially Present (cfu/mL)	Microbes Initially Present (Log ₁₀)	Log ₁₀ Reduction	Percent Reduction
"Reflexx" Lot # 037-25-10032008	30 seconds	1 ounce/6gallons (1:768)	685	7.6 x 10 ⁷	7.88	5.04	>99.999%
"Reflexx" Lot # 037-25-21052008	30 seconds	1 ounce/6gallons (1:768)	680	7.6 x 10 ⁷	7.88	5.05	>99.999%
"Reflexx" Lot # 037-25-28052008	30 seconds	1 ounce/6gallons (1:768)	680	7.6 x 10 ⁷	7.88	5.05	>99.999%

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Table 3: Raw Data Results for *Escherichia coli*

Test Substance	Test Dilution (v/v)	Exposure Time	Flask	Plate Counts (CFU/plate)					
				Number Surviving		Number Controls (10 ⁶) (Microbes Initially Present)			
				10 ⁻¹	10 ⁻²	Flask A	Flask B		
"Reflexx" Lot # 037-25-10032008	1 ounce/6gallons (1:768)	30 seconds	Flask A	0,0,0,0	0,0,0,0				
			Flask B	0,0,0,0	0,0,0,0				
		60 seconds	Flask A	0,0,0,0	0,0,0,0				
			Flask B	0,0,0,0	0,0,0,0				
"Reflexx" Lot # 037-25-21052008	1 ounce/6gallons (1:768)	30 seconds	Flask A	0,0,0,0	0,0,0,0			8.1 x 10 ⁷ 8.2 x 10 ⁷ 8.8 x 10 ⁷ 8.7 x 10 ⁷	8.7 x 10 ⁷ 8.7 x 10 ⁷ 8.4 x 10 ⁷ 8.9 x 10 ⁷
			Flask B	0,0,0,0	0,0,0,0				
		60 seconds	Flask A	0,0,0,0	0,0,0,0				
			Flask B	0,0,0,0	0,0,0,0				
"Reflexx" Lot # 037-25-28052008	1 ounce/6gallons (1:768)	30 seconds	Flask A	0,0,0,0	0,0,0,0				
			Flask B	0,0,0,0	0,0,0,0				
		60 seconds	Flask A	0,0,0,0	0,0,0,0				
			Flask B	0,0,0,0	0,0,0,0				
								Avg. of Flask A and B 8.6 x 10 ⁷ = 7.93 Log	

Table 4: Calculated Results for *Escherichia coli* (cfu/mL) by Lot, Exposure, and corresponding Percent and Log₁₀ Reduction

Test Substance / Lot #	Exposure Time	Test Dilution (v/v)	Average Number Surviving (cfu/mL)	Microbes Initially Present (cfu/mL)	Microbes Initially Present (Log ₁₀)	Log ₁₀ Reduction	Percent Reduction
"Reflexx" Lot # 037-25-10032008	30 seconds	1 ounce/6gallons (1:768)	<10	8.6 x 10 ⁷	7.93	≥6.93	>99.999%
"Reflexx" Lot # 037-25-21052008	30 seconds	1 ounce/6gallons (1:768)	<10	8.6 x 10 ⁷	7.93	≥6.93	>99.999%
"Reflexx" Lot # 037-25-28052008	30 seconds	1 ounce/6gallons (1:768)	<10	8.6 x 10 ⁷	7.93	≥6.93	>99.999%

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Table 5: Neutralization Effectiveness and Neutralization System Toxicity Control Results

Test Substance and Test	<i>Staphylococcus aureus</i>			<i>Escherichia coli</i>		
	Test Tube A (cfu/mL)	Test Tube B (cfu/mL)	Inoculum (cfu/mL)	Test Tube A (cfu/mL)	Test Tube B (cfu/mL)	Inoculum (cfu/mL)
Neutralization Effectiveness Control "Reflexx" Lot # 037-25-10032008	13	17	19, 16	34	40	41, 44
Neutralization Effectiveness Control "Reflexx" Lot # 037-25-21052008	12	19		42	35	
Neutralization Effectiveness Control "Reflexx" Lot # 037-25-28052008	17	16		38	39	
Neutralization System Toxicity Control (Neutralizer Broth)	18	16		46	41	

Table 6: Sterility Control Results

Reagents and Lot #'s	Result
AOAC Hard Water Lot # C-1922	Sterile
Phosphate Buffer Dilution Water Lot # E-224	Sterile
Tryptone Glucose Extract Agar (TGEA) Lot # E-352, F-29, 30	Sterile, Sterile, Sterile
Neutralizer Broth (Phosphate Buffer Dilution Water containing 57.2 units catalase / mL and 0.05% Sodium thiosulfate) Lot # E-224	Sterile

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