NON-GLP STUDY REPORT

STUDY TITLE

OECD Quantitative Method for Testing Antimicrobial Products Against Spores of *Clostridium difficile* (ATCC 43598) on Inanimate, Hard, Non-porous Surfaces

Test Organism:

Clostridium difficile - spore form (ATCC 43598)

PRODUCT IDENTITY

Envirocleanse-A
Lot 1 #090619 and Lot 2 #090919

AUTHOR

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STUDY COMPLETION DATE

November 25, 2019

PERFORMING LABORATORY

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SPONSOR

Envirocleanse, LLC
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PROJECT NUMBER

A28652

This study was not performed under
EPA Good Laboratory Practice Regulations
(40 CFR Part 160)

STUDY REPORT

GENERAL STUDY INFORMATION

Study Title: OECD Quantitative Method for Testing Antimicrobial Products Against Spores of *Clostridium difficile* (ATCC 43598) on Inanimate, Hard, Non-porous Surfaces

Project Number: A28652

Protocol Number: ECL01082919.OECDCD

TEST SUBSTANCE IDENTITY

Test Substance Name: Envirocleanse-A

Batch/Lot(s): Lot 1 #090619 and Lot 2 #090919

STUDY DATES

Date Sample Received: September 12, 2019
Study Initiation Date: October 14, 2019
Experimental Start Date: October 14, 2019
Experimental End Date: October 29, 2019
Study Completion Date: November 25, 2019

Test Organism	ATCC #	Growth Medium	Incubation Parameters
Clostridium difficile - spore form	43598	CDC Anaerobic Blood Agar	35-37°C, anaerobic

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

TEST HISTORY

Testing performed on October 14, 2019, resulted with an invalid neutralization control for Lot 2 #090919, however, Lot 1 #090619 resulted in all valid controls and passing results. Lot 2 #090919 was deemed invalid for controls and test results. Testing was repeated on October 24, 2019 for Lot 2 #090919 and resulted with all valid controls and test results.

Test Substance Dilution:	Ready to use
Exposure Time:	10 minutes
Exposure Temperature:	Room temperature (20°C)
Number of Carriers Tested/Lot:	10 test carriers per batch
Organic Soil Load:	0.25% Bovine Serum Albumin, 0.08% Bovine Mucin and 0.35% Yeast Extract (final concentrations) [Lot 1 #090619] No organic soil load required [Lot 2 #090919]
Test Substance Neutralizer:	Phosphate Buffered Saline + 0.1% Tween 80 + 0.1% Sodium Thiosulfate (Test Date: 10/14/19) Phosphate Buffered Saline + 0.1% Tween 80 + 0.5% Sodium Thiosulfate (Test Date: 10/24/19)
NaOCl Neutralizer	Phosphate Buffered Saline + 0.1% Tween 80 + 0.1% Sodium Thiosulfate
Agar Plate Medium:	BHIY-HT Agar

EXPERIMENTAL DESIGN

A film of purified *Clostridium difficile* spores was dried on the surface of brushed stainless steel AISI #430 disks (diameter ≈ 1 cm, thickness ≈ 0.8 mm). Each inoculated and dried disk was placed into a vial with the contaminated side facing up. Fifty (50) µL of test substance was applied to the center of the disk. Care was taken to ensure that the entire inoculated area on the disk was covered with test substance. The treated disk was held at the exposure temperature for the Sponsor specified exposure time. After exposure, the carriers were neutralized and assayed for survivors. Appropriate culture purity, carrier sterility, organic soil load sterility, neutralizer sterility, carrier population and neutralization confirmation controls containing the organic soil load (for Lot 1 #090619 only) were performed.

Per Sponsor's direction, the study was not required to be conducted under US EPA 40 CFR Part 160 or US FDA 21 CFR Part 58.

STUDY RESULTS

TABLE 1: CONTROL RESULTS

The following results from controls confirmed study validity:

Type of Control		Results
		***Clostridium difficile* - spore form (ATCC 43598)**
Culture Purity		Pure
Carrier Sterility		No Growth
Neutralizer Sterility		No Growth
Phosphate Buffered Saline Sterility		No Growth
Phosphate Buffered Saline + 0.1% Tween 80 Sterility		No Growth
Organic Soil Load Sterility (7/26/19)	Yeast Extract	No Growth
	Bovine Serum Albumin	No Growth
	Bovine Mucin	No Growth

TABLE 2: CARRIER POPULATION CONTROL RESULTS

Test Organism: *Clostridium difficile* - spore form (ATCC 43598)					
Test Date: 10/14/19 (with soil)					
Carrier #	**Dilution Factor**		**CFU/Carrier**	**Log_{10}**	**Geometric Mean (Average Log_{10})**
	10^{-4}	**10^{-5}**			
1	T	35	3.5×10^6	6.54	3.39×10^6 (6.53)
2	T	32	3.2×10^6	6.51	
3	T	34	3.4×10^6	6.53	
Test Date: 10/14/19 (without soil)					
Carrier #	**Dilution Factor**		**CFU/Carrier**	**Log_{10}**	**Geometric Mean (Average Log_{10})**
	10^{-4}	**10^{-5}**			
1	T	49	4.9×10^6	6.69	5.62×10^6 (6.75)
2	T	58	5.8×10^6	6.76	
3	T	64	6.4×10^6	6.81	
Test Date: 10/24/19 (without soil)					
Carrier #	**Dilution Factor**		**CFU/Carrier**	**Log_{10}**	**Geometric Mean (Average Log_{10})**
	10^{-4}	**10^{-5}**			
1	T	61	6.1×10^6	6.79	5.25×10^6 (6.72)
2	T	53	5.3×10^6	6.72	
3	T	46	4.6×10^6	6.66	

CFU = Colony Forming Unit
T = Too Numerous To Count (>200 colonies)

TABLE 3: 1,500 ppm SODIUM HYPOCHLORITE TEST CONTROL

Test Organism: *Clostridium difficile* - spore form (ATCC 43598)							
NaOCl Titration: 1436 ppm							
Test Date: 10/14/19							
Carrier #	**Colony Forming Units**			**CFU/ Control Carrier (Log_{10})**	**Average Log_{10} of Control Carriers**	**Log_{10} Reduction**	**Pass/ Fail***
	10^{-3}	**10^{-4}**	**10^{-5}**				
1	89	11	2	1.0×10^5 (5.00)	5.26	1.27	Pass
2	118	13	4	1.35×10^5 (5.13)			
3	T	41	5	4.6×10^5 (5.66)			
NaOCl Titration: 1450 ppm							
Test Date: 10/24/19							
Carrier #	**Colony Forming Units**			**CFU/ Control Carrier (Log_{10})**	**Average Log_{10} of Control Carriers**	**Log_{10} Reduction**	**Pass/ Fail***
	10^{-3}	**10^{-4}**	**10^{-5}**				
1	T	33	3	3.6×10^5 (5.56)	5.57	1.15	Pass
2	T	45	2	4.7×10^5 (5.67)			
3	T	28	3	3.1×10^5 (5.49)			

CFU = Colony Forming Units
T = Too numerous to count (>200 CFU)
*The passing criteria is established as a mean <3.0 Log_{10} reduction when tested against 1,500 ppm NaOCl on the day of testing.

TABLE 4: NEUTRALIZATION CONFIRMATION CONTROL RESULTS

Test Organism: *Clostridium difficile* - spore form (ATCC 43598)					
Test Date: 10/14/19					
Organism Dilution used: 10^{-5}					
Control Type	**Test Substance**	**Vial**	**CFU**	**Percent Recovery**	**Pass/ Fail***
Treatment 1: (Titer Control)	Not Applicable	1	36	Not Applicable	Pass
Treatment 2: (Neutralizer Toxicity Control)	Not Applicable	1	32	89%	Pass
Treatment 3: (Neutralizer Effectiveness)	Envirocleanse-A Lot 1 #090619	1	30	83%	Pass
Test Date: 10/24/19					
Organism Dilution used: 10^{-5}					
Control Type	**Test Substance**	**Vial**	**CFU**	**Percent Recovery**	**Pass/ Fail***
Treatment 1: (Titer Control)	Not Applicable	1	45	Not Applicable	Pass
Treatment 2: (Neutralizer Toxicity Control)	Not Applicable	1	50	111%	Pass
Treatment 3: (Neutralizer Effectiveness)	Envirocleanse-A Lot 1 #090919	1	54	120%	Pass

CFU = Colony Forming Unit
*The passing criteria is established as an average of 20-200 CFU for Treatment 1 and a percent recovery of ≥50% for Treatments 2 and 3.

TABLE 5: TEST RESULTS FOR Envirocleanse-A Lot 1 #090619

Test Organism: *Clostridium difficile* - spore form (ATCC 43598)			
Carrier #	**CFU Results**		**CFU/Carrier (Log_{10})**
	10^0 (Vial – 9 mL)	**10^{-1} (10 mL)**	
1	T	T	$>2.00 \times 10^3$ (>3.30)
2	T	T	$>2.00 \times 10^3$ (>3.30)
3	T	T	$>2.00 \times 10^3$ (>3.30)
4	T	T	$>2.00 \times 10^3$ (>3.30)
5	T	T	$>2.00 \times 10^3$ (>3.30)
6	T	T	$>2.00 \times 10^3$ (>3.30)
7	T	T	$>2.00 \times 10^3$ (>3.30)
8	T	T	$>2.00 \times 10^3$ (>3.30)
9	T	T	$>2.00 \times 10^3$ (>3.30)
10	T	T	$>2.00 \times 10^3$ (>3.30)
Geometric Mean of Test Carriers (Average Log_{10})			$>2.00 \times 10^3$ (>3.30)
Percent Reduction (Log_{10} Reduction)			<99.9410% (<3.23)

CFU = Colony Forming Units
T = Too Numerous To Count (>200 colonies)

TABLE 6: TEST RESULTS FOR Envirocleanse-A Lot 2 #090919

Test Organism: *Clostridium difficile* - spore form (ATCC 43598)			
Carrier #	**CFU Results**		**CFU/Carrier (Log_{10})**
	10^0 (Vial – 9 mL)	**10^{-1} (10 mL)**	
1	0	0	$<1 \times 10^0$ (<0.00)
2	0	0	$<1 \times 10^0$ (<0.00)
3	0	0	$<1 \times 10^0$ (<0.00)
4	0	0	$<1 \times 10^0$ (<0.00)
5	0	0	$<1 \times 10^0$ (<0.00)
6	0	0	$<1 \times 10^0$ (<0.00)
7	0	0	$<1 \times 10^0$ (<0.00)
8	0	0	$<1 \times 10^0$ (<0.00)
9	0	0	$<1 \times 10^0$ (<0.00)
10	40	3	4.3×10^1 (1.63)
Geometric Mean of Test Carriers (Average Log_{10})			$<1.46 \times 10^0$ (<0.163)
Percent Reduction (Log_{10} Reduction)			>99.9999% (>6.56)

CFU = Colony Forming Units
A value of <1 was used in place of zero for calculation purposes.

CONTROL RESULTS

The results of controls run for culture purity, organic soil sterility, neutralizer sterility, carrier sterility, Phosphate Buffered Saline sterility, Phosphate Buffered Saline + 0.1% Tween 80 sterility, carrier population and neutralization confirmation controls were all acceptable.

ANALYSIS

Envirocleanse-A (Lot 1 #090619 and Lot 2 #090919), ready to use, demonstrated a <99.9410% (<3.23 Log_{10}) and a >99.9999% (>6.56 Log_{10}) log reduction, respectively, of *Clostridium difficile* - spore form (ATCC 43598) following a 10 minute exposure time when tested at room temperature (20°C) in the presence of a 0.25% bovine serum albumin, 0.08% bovine mucin and 0.35% yeast extract organic soil load (soil was used for Lot 1 #090619 only).

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11/25/19
Date