CONFIDENTIAL FINAL REPORT

SPONSOR: Envirocleanse LLC
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SPONSOR'S REPRESENTATIVE: Phil Bellan

STUDY TITLE: Quantitative Carrier Test to Determine Sporicidal Efficacy Using *Clostridium difficile* Spores

STUDY IDENTIFICATION: MicroBioTest Project No. 668-110
*Refer to signed protocol (Protocol No. 668.1.03.22.16)

TEST AGENT :	LOT NO.	RECEIVED DATE	DS NO.
Anolite (Envirocleanse A)	#3	04/04/16	G193

ACTIVE INGREDIENT(S): Hypochlorous acid

DILUTION(S): Ready to Use

NEUTRALIZER: Letheen Broth containing 0.5% Sodium Thiosulfate

CHALLENGE ORGANISMS: *Clostridium difficile,* ATCC 43598

ORGANIC LOAD: Using the following ratios per 500 µLof the final test spore suspension:

- 340 µL of the prepared spore suspension
- 25 µL BSA
- 100 µL mucin
- 35 µL yeast extract

CONTACT TEMPERATURE AND % RELATIVE HUMIDITY (RH): 22C and 34-35% RH

CONTACT TIME: 9 minutes 58 seconds

MicroBioTest, Division of Microbac Laboratories, Inc.
105 Carpenter Drive | Sterling, VA 20164 | 703.925.0100 p | 703.925.9366 f | www.microbac.com

RESULTS: Results are presented in Tables 1-3. The titer of the test inoculum was 4.8 x 10^8 colony-forming units (CFU) per mL. The challenge microorganism was confirmed by colony morphology and spore stain to be consistent with *Clostridium difficile.*The spore concentration in test spore suspension was observed to be ≥ 95% using wet-mount technique using phrase contrast microscopy. The sterility controls exhibited no growth.

The Log_{10} reduction was calculated by the following formula:

Log_{10} (Average Carrier counts (CFU/carrier)) – Log_{10} (Test result (CFU recovered)) = Log_{10} Reduction

Table 1
Carrier Counts
Results Expressed as Average Colony Forming Units (CFU) per carrier and Average CFU per carrier

Replicate	Log_{10} CFU/Carrier	Mean CFU/Carrier
1	6.18	6.30
2	6.37	
3	6.36	

Table 2
Test Results
Results Expressed as Number of CFU recovered per Replicate and Log_{10} Reduction

Test Agent	Replicate	Log_{10} CFU/Carrier	Mean Log_{10} CFU/Carrier	Log_{10} Reduction
# 3	1	0.30	0.28	6.02
	2	0.48		
	3	-0.30		
	4	-0.30		
	5	0.60		
	6	0.70		
	7	-0.30		
	8	0.00		
	9	-0.30		
	10	0.30		

Table 3
Neutralizer Effectiveness Results – Disinfectant II Mod.3

Controls	Mean CFU/Carrier	Percent Recovery
NE Titer Control	40	Not applicable
NE Control	34	85.7143%
NE Toxicity Control	34	86.5546%

*Percent Recovery calculated using Average Log_{10} Recovered based on the following calculation: (Neutralizer Toxicity or Effectiveness Control / Titer Control) * 100

Note: The Average Log_{10} Recovered for the Neutralizer Toxicity and Neutralizer Effectiveness Controls were 50% of the Titer Control. Therefore, the Neutralizer was determined to be non-toxic and effective.

CONCLUSIONS:

According to the EPA, the average of ten test carriers must achieve at least a 6-Log_{10} reduction from the average CFU/carrier of the carrier count controls.

When tested as described, Anolite (Envirocleanse A) passed the test when *Clostridium difficile,* containing the required organic load, was exposed to the test agent for 9 minutes 58 seconds at 22C.

All of the controls met the criteria established for a valid test. These conclusions are based on observed data.

Saha

Study Director: Shirshendu Saha

4/27/16

Date