Certificate of Antimicrobial Testing

Study Title

ASTM E3218 Quantitative Method for Evaluating the Efficacy of Microbicides Against Spores of *Clostridioides difficile* (ATCC 43598) on Hard, Non-porous Surfaces

Study Identification Number

NG22305

Author

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Study Completion Date

31JAN2025

Testing Facility

Microchem Laboratory
1700 Chisholm Trail Road
Round Rock, TX 78681

Study Sponsor

Envirocleanse LLC
23403 Clay Rd.
Katy, TX 77493

Study Dates

Experimental Start Date/Time:	19MAR2025 / 1153
Experimental Termination Date/Time:	24MAR2025 / 1237

Test System

Test Microorganism(s):
Clostridioides difficile ATCC 43598

Test Substance:

Test Substance: Enviocleanse A Lot: 011725
Test Substance Receipt Date: 24JAN2025

Test Parameters

Test Substance Dilution: None

Organic Soil Load: No organic soil load incorporated into test inoculum.

Inoculum volume: 0.01 mL

Carrier Type: AISI #304 stainless steel disks

Agar Medium: Pre-reduced BHIY-HT agar

Number of Carriers: 10 per contact time

Contact Times: 2 minutes, 3 minutes

Exposure Temperature: 22±2°C

Neutralizer: Dey/Engley Broth (10.0 mL)

Results

Table 1: Test System Control Carrier Enumeration Results

Test Microorganism	Contact Time	Test or Control Substance	Carrier Replicate	Carrier Enumeration (CFU/Carrier)	Log_{10} Density	Mean Log_{10} Density
Clostrioides difficile ATCC 43598	3 minutes	PBS-T Control Substance	1	5.30×10^6	6.72	6.32
			2	1.90×10^6	6.28	
			3	9.20×10^5	5.96	

CFU = colony forming unit

Table 2: Test Carrier Enumeration Results

Test Microorganism	Contact Time	Test or Control Substance	Carrier Replicate	Carrier Enumeration (CFU/Carrier)	Mean Log_{10} Reduction
Clostrioides difficile ATCC 43598	2 minutes & 3 minutes	Envirocleanse A Lot: 011725	1	0*	>6.32[1]*
			2	0*	
			3	0*	
			4	0*	
			5	0*	
			6	0*	
			7	0*	
			8	0*	
			9	0*	
			10	0*	

[1]Per the method, the log reduction is reported as greater than the log density of the PBS-T Control Carriers if all counts are zero for all dilutions performed.
**For both contact times tested.*

Performance Criteria

The test substance must demonstrate a ≥6 log_{10} reduction over the parallel control to meet *C. difficile* sporicidal efficacy requirements.