FINAL REPORT

PROTOCOL
EPA SOP MB-06-09: Germicidal Spray Products as Disinfectants (Modified)

PRODUCT TESTED
Envirocleanse A

EMSL ORDER NUMBER
151900115

TESTING LABORATORY
EMSL Analytical, Inc.
5950 Fairbanks North Houston Rd.
Houston TX 77040
Phone: (713) 686-3635
Web: www.emsl.com

SPONSOR
Envirocleanse Inc.
12621 W. Airport Blvd., Suite 200
Sugarland, TX 77478
Contact: Scott Mack
Phone: 713-234-2134
Email: smack@eco-enviro.com

STUDY START DATE
January 7, 2019

STUDY COMPLETION DATE
March 21 2019

Test Summary

Project Title: Antibacterial efficacy of Envirocleanse A against *Mycobacterium bovis*.

Study Methods: EPA SOP MB-06-09 (2017)

Product Tested: Envirocleanse A, Lot #020419

Sponsor: Envirocleanse, Inc.

Test Conditions:

Challenge Organisms:

1. *Mycobacterium bovis,* ATCC35732

Broth used: Middlebrook 7H9 Broth-T with 0.1% (w/v) sodium thiosulfate

Contact time: 10 minutes

Contact Temperature: Room temperature

Study Dates and Facilities

All analytical testing was performed at EMSL Analytical, Inc. in Houston, Texas from 1/7/2019 to 3/21/2019.

Record Retention

All raw data and a copy of the final report will be archived and stored by EMSL Analytical, Inc. for 5 years.

Objectives

To determine the efficacy of the germicidal spray, Envirocleanse A, against *Mycobacterium bovis* on inanimate, hard, non-porous surfaces after 10 minutes of contact time at room temperature.

Experimental Summary

This method used glass slide carriers to represent a hard, non-porous surface. Each carrier received 10 µL of microbial suspension. The inoculum was dried and sprayed with the test product. The contact time was allowed to elapse, the excess test product was drained from each carrier without touching the Petri dish or filter paper then the carrier was placed in the neutralizer broth. The neutralized carriers were vortexed, and the resulting suspension was incubated to determine if any viable bacteria remained. For viability controls, one dried inoculated but untreated carrier was placed into individual tubes of Middlebrook 7H9 Broth-T with 0.1% (w/v) sodium thiosulfate.

Materials

Test product: One lot of Envirocleanse A. The test product was tested as supplied by the sponsor. The total chlorine concentration for lot# 020419 was 800 ppm.

The sponsor assured EMSL Analytical, Inc. testing facility management that the test product has been appropriately tested for identity, strength, purity, stability, and uniformity as applicable.

Materials supplied by EMSL Analytical, Inc. including, but not limited to:

Apparatus

As specified in MB-06-09.

Media and Reagents

As specified in MB-06-09 and additionally:
Middlebrook 7H9 Broth-T for initial stock cultures
Middlebrook 7H10 Agar for initial microbe plating
Middlebrook 7H9 Broth-T with 0.1% (w/v) sodium thiosulfate broth for recoveries

Test Method

EPA SOP MB-06-09 was followed for this testing with modifications to the test organisms and culture media appropriate for each microbe; as requested by the sponsor.

Procedure

Preparation of Bacteria – Using stock cultures, Middlebrook 7H9 Broth with Tween 80 was inoculated, vortexed and incubated at 36±1°C for 21 days. One monthly transfer was prepared prior to the inoculation of a final test culture.

To generate test cultures, a sufficient number of 20×150 mm tubes containing 10 mL growth medium (e.g., Middlebrook 7H9 Broth-T) were inoculated with 10 µL per tube of the 21 day culture, then vortexed to mix. Tubes were incubated 21 days at 36±1°C.

Inoculation of Test Pieces – 10 carriers were inoculated for treatment, 3 for control carrier counts, and 3 for viability controls.

For the bacteria tested, a vortex-style mixer was used to mix the test cultures for 3-4 seconds and then let stand for 10 minutes at room temperature before continuing. The upper portion of each culture (e.g., upper ¾) was removed, leaving behind any debris or clumps, and transferred to a sterile container. Cultures were pooled in the flask and swirled to mix. The absorbance was measured and recorded using a BIOLOG Turbidimeter. Sterile broth medium was used as a blank for the turbidimeter. The test culture was used for carrier inoculation within 30 minutes.

One mL of organic soil load was added then swirled to mix. An aliquot (e.g., ~10 mL) of the final test culture was transferred into a sterile tube for carrier inoculation. A pipette was used to transfer 0.01 mL of the culture to the sterile test carrier in the Petri dish. Immediately, the inoculum was spread uniformly using a sterile loop and the dish covered. Carriers were then dried in an incubator at 36±1°C for 30-40 min. Efficacy testing was performed within two hours of drying.

Enumeration of viable bacteria from carriers (control carrier counts)
As described in MB-06-09

Treatment Procedure
As described in MB-06-09

Experimental Results:

Table 1: Enumeration of viable bacteria from carriers (control carrier counts).

Organism	Counts Prior to Testing (Pooled CFU/mL)	Counts After Testing (Pooled CFU/mL)
Mycobacterium bovis	6.80×10^4	7.40×10^4

Table 2: Sterility control and viability control results (Growth Presence/Absence).

Organism	Sterility (Growth +/0)		Viability (Growth +/0)		
	Rep1	Rep2	Rep1	Rep2	Rep3
Mycobacterium bovis	0	0	+	+	+

Sterility control: Growth should not occur in any of the tubes.
Viability control: Growth should occur in all tubes.

Table 3: Germicidal spray efficacy of Envirocleanse A against seven bacterial test agents.

Organism	**Envirocleanse A Lot** 020419 (10 replicates)	
	Tests with Growth	Tests with No Growth
Mycobacterium bovis	0	10

To demonstrate efficacy growth should not occur in any of the tubes.

Conclusions:

The Envirocleanse A spray was effective against *Mycobacterium bovis.*

References

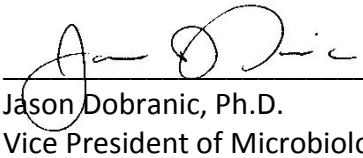
SOP-MB-06-09: Procedure for Germicidal Spray Products as Disinfectants (GSPT): Testing of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Salmonella enterica*, Revised:09-29-17.

Signatures

Study Performed by:

Muntaha Ramadi, Ph.D. — 3/21/2019
Microbiologist — Date

Report Issued by:



Jason Dobranic, Ph.D. — 3/21/2019
Vice President of Microbiology & Life Sciences — Date
Study Director

Reviewed by:

Terri Lawrence — 3/21/2019
Microbiology Laboratory Manager/Quality Manager — Date