



# FINAL REPORT

## PROTOCOL

AOAC 961.02  
Germicidal Spray Products as Disinfectants

EMSL ORDER NUMBER  
242006329

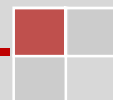
TESTING LABORATORY  
EMSL Analytical, Inc.  
29 North Plains HWY Unit #4  
Wallingford, CT 06492  
Phone: (203) 284-5948  
Web: [www.emsl.com](http://www.emsl.com)

SPONSOR  
Toppen Health  
417 Central Park Drive  
Sanford, FL 32771  
Contact: Ron Psimas

STUDY START DATE  
October 08, 2020

STUDY COMPLETION DATE  
October 29, 2020

Test Summary





Project Title: Efficacy testing of an antibacterial hard surface disinfectant.

Study Methods: AOAC 961.02 Germicidal Spray Product as Disinfectant Method

Product Tested: Toppen Dental Dolphin Pods- Multi-purpose Effervescent Tablets

Sponsor: Toppen Health

Test Conditions:

Challenge Organisms:

1. Methicillin-resistant *Staphylococcus aureus* ATCC 33591
2. *Escherichia coli* ATCC 8537
3. *Pseudomonas aeruginosa* ATCC 9027
4. *Mycobacterium bovis* ATCC 35732

Broth used: TAT Broth (Trypton-Azolectin-Tween) Neutralizing broth

Contact time: 1 and 2 minutes.

Contact Temperature: Room temperature

Study Dates and Facilities

All analytical testing was performed at EMSL Analytical, Inc. in Wallingford, CT from 10/08/2020 to 10/29/2020.

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

Toppen Dental Dolphin Pods- Multi-purpose Effervescent Tablets against *E. coli*, *P. aeruginosa*, *S. aureus* (MRSA), and *M. bovis* on inanimate, hard, non-porous surfaces after 1, 2 minutes of contact time at room temperature.

Experimental Summary

The testing procedure was designed after discussions between EMSL Analytical Inc., the testing company, and the client, Toppen Health, Inc. The testing procedure is based on AOAC 961.02 test method, with the testing conducted on Toppen Dental Dolphin Pods- Multi-purpose Effervescent Tablets (supplied by client), to demonstrate its effectiveness at killing *E. coli*, *P. aeruginosa*, *S. aureus*(MRSA), and *M. bovis*. The Toppen Dental Dolphin Pods-Multi-purpose Effervescent Tablets were prepared according to client's instructions by filling the bottle with 24 ounces of water and adding one tablet. The tablet was allowed to dissolve in water before putting on the spray head.

Procedure:

Culture Preparation:

*E. coli*, *P. aeruginosa*, and *S. aureus* (MRSA) from stock culture were plated onto Tryptic Soy Agar and incubated at 35°C for 24 hours. *M. bovis* from stock culture was plated onto Middlebrook Agar at 35°C for 10 days. After incubation a bacterial suspension was prepared for each of the bacteria by taking one 10µL loop of the test bacteria into 10 mL of Normal Saline 0.85% until a 10<sup>8</sup> solution of cells was created with initial concentrations as follows: *E. coli* with a 9.1 x 10<sup>8</sup>



concentration, *S. aureus- MRSA* with a  $6.8 \times 10^8$  concentration, *P. aeruginosa* with a concentration of  $8.1 \times 10^8$ , and *M. bovis* with a  $2.8 \times 10^8$  concentration.

**Qualitative Test:**

Sterile glass slide carriers were inoculated with 10 µL of each of the microorganism suspensions and incubated to air dry at 35°C for 40 minutes. Inoculated test carriers were individually sprayed with Toppen Dental Dolphin Pods- Multi-purpose Effervescent Tablets, exposed for 1 and 2 minutes for all bacteria strain and then neutralized in 10 mL of TAT neutralizing broth.

The neutralized subculture and controls for *E. coli*, *P. aeruginosa*, and *S. aureus-MRSA* were incubated for 48 hours at 35.0°C. The neutralized subcultures and controls for *M. bovis* were incubated for 10 days at 35°C. Viability controls of each bacterium were added to the corresponding subculture medium. A representative uninoculated carrier was added to the neutralizing subculture medium. All the subculture medium containing the carriers were visually examined for growth or no growth for all the bacterium types. The subculture medium containing the uninoculated carrier was incubated and examined for lack of growth.

**Experimental Results:**

<b>Table 1. Control Results</b>				
Type of Control	Results			
	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>Methicillin-resistant Staphylococcus aureus</i>	<i>Mycobacteria bovis</i>
Purity Control	Pure	Pure	Pure	Pure
Viability Controls	Growth	Growth	Growth	Growth
Neutralizing Subculture Medium Sterility Control	No Growth			
Carrier Sterility Control	No Growth			

<b>Table 2. Efficacy of Toppen Dental Dolphin Pods- Multi-purpose Effervescent Tablets Test Results</b>			
Time Points (minutes)	Test Organism	Number of Carriers	
		Exposed	Showing Growth*
1	<i>Escherichia coli</i> ATCC 8537	10	0
	<i>Pseudomonas aeruginosa</i> ATCC 9027	10	0
	<i>Methicillin-resistant Staphylococcus aureus</i> ATCC 33591	10	0
	<i>Mycobacteria bovis</i> ATCC 35732	10	0
2	<i>Escherichia coli</i> ATCC 8537	10	0
	<i>Pseudomonas aeruginosa</i> ATCC 9027	10	0
	<i>Methicillin-resistant Staphylococcus aureus</i> ATCC 33591	10	0
	<i>Mycobacteria bovis</i> ATCC 35732	10	0

\*Number of carrier showing growth of the test organism.




**Conclusion:**

The Toppen Dental Dolfin Pods- Multi-purpose Effervescent Tablets were able to inactivate all bacteria listed above resulting in complete disinfection on the 10 carriers per bacteria type.

**Signatures**

Study Performed by:

 10/28/2020  
Gloria V. Oriol-Aguilar  
Microbiology Laboratory Director  
Date

 10/20/2020  
Evan Buckley  
Microbiology Quality Manager  
Date

 10/26/2020  
Sherly Santiago  
Microbiologist  
Date

Report Issued by:

 11/06/2020  
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Date