

Microconsult, Inc

Determination of the Antimicrobial Efficacy of a Test Product Using the Minimum Inhibition Concentration Method.

Purpose:

The purpose of this study is to evaluate the antimicrobial effectiveness of a test product using the Minimum Inhibition Concentration Method.

Scope:

The in vitro antimicrobial effectiveness of a test product utilizing nine dilutions of the test material in tryptic soy broth will be determined.

Test Material:

Test Product: 8 oz Health Care Hand Sanitizing Solution

Lot Number: 746.015

Manufacturer: Dr.'s Solutions, LLC

Equipment and Supplies:

Pipetter 1.0 mL Capacity

Pipetter 0.1 mL Capacity

Bunsen Burner

Incubator 35⁰ +/- 2⁰C

Vortex Mixer

Sterile 10.0 mL Serological Pipettes

Sterile Dilution Tubes

Sterile Polystyrene Petri Dishes

Sterile 1.0 mL Capacity Pipette Tips

Sterile 0.1 mL Capacity Pipette Tips

Test Microorganisms: Manufacturer: Quality Technologies

Microorganism	ATCC#	Lot#
<i>Escherichia coli</i>	8739	5315
<i>Pseudomonas aeruginosa</i>	9027	5318
<i>Salmonella cholerasuis</i>	10708	4949
<i>Serratia marcescens</i>	14756	5779
<i>Listeria monocytogenes</i>	7644	5389
<i>Lactobacillus fermentum</i>	9338	5019
<i>Bacillus subtilis</i>	6633	5521
<i>Candida albicans</i>	10231	5066

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Test Solutions and Media:

Sterile Tryptic Soy Broth (TSB), (Alpha Bioscience Inc., Lot D02-02, exp. 05/2005)

Sterile Phosphate Buffered Saline (PBS), (Microconsult Inc., Lot 100203).

Tryptic Soy Agar (TSA), (Alpha Bioscience Inc., Lot B03-24, exp. 03/2006)

Test Methods:

Prepare dilutions of the test material by placing one to nine grams of test product into consecutive test tubes that contain sterile tryptic soy broth to prepare dilutions of the test material from ten percent to ninety percent of the original product. Prepare the dilutions for each of the eight test microorganisms.

Inoculum Preparation

Prepare each test microorganism by streaking the test organism to a tryptic soy agar plate. Incubate at 35C +/- 2C for approximately twenty-four hours. Remove an aliquot of each test organism and place into sterile PBS. Further dilute in PBS to make an approximate count between 1×10^5 and 1×10^6 cfu/ml. The inoculum will consist of 0.1 mL of test organism into ten mLs of diluted product. The final concentration of each inoculum should be between 1×10^3 and 1×10^4 cfu/ml of diluted product.

Test Period

Following inoculation of the test tubes containing the diluted product, incubate at ambient room temperature, 25C +/- 2C for twenty to twenty four hours. Perform serial dilutions to determine the Minimum Inhibitory Concentration. Incubate plates forty eight to seventy two hours at 35C +/- 2C. Remove plates and count visible colonies.

Method of Analysis:

Data Collection

Determine the MIC by calculating the log reduction of the viable microorganisms following forty eight to seventy two hours of incubation. The average of two dilutions will be utilized for the log reduction. The lowest concentration of the diluted test product which inhibits growth is the minimum inhibitory concentration.

Findings and Conclusions:

There were no detectable viable microorganisms remaining at any of the dilutions ranging from ten to ninety percent of the test product. The data shows that the minimum inhibitory concentration is actually the minimum lethal concentration for the test microorganisms utilized at the levels inoculated. The minimum lethal dose is ten percent of the test product.

MicroDine	Lot # 746.015	MIC Initiated 10-3-03					Plated 10-5-03			Results 10-7-03		
		90%	80%	70%	60%	50%	40%	30%	20%		10% Log Reduc	
Inoculum Levels <i>Escherichia coli</i> 15600		1	1	1	1	1	1	1	1	1	1	4.193125
<i>Pseudomonas aeruginosa</i> 18720		1	1	1	1	1	1	1	1	1	1	4.272306
<i>Salmonella cholerasuis</i> 5200		1	1	1	1	1	1	1	1	1	1	3.716003
<i>Listeria monocytogenes</i> 5200		1	1	1	1	1	1	1	1	1	1	3.716003
<i>Serratia marcescens</i> 5720		1	1	1	1	1	1	1	1	1	1	3.757396
<i>Bacillus subtilis</i> 14040		1	1	1	1	1	1	1	1	1	1	4.147367
<i>Candida albicans</i> 48360		1	1	1	1	1	1	1	1	1	1	4.684486
<i>Lactobacillus fermentum</i> 4680		1	1	1	1	1	1	1	1	1	1	3.670246

Completed By: C. J. [Signature] Date: 10-7-03

Reviewed By: W. J. [Signature] Date: 10-7-03