

MICROCONSULT, INC.

Microbiological Testing Laboratory

KILL RATE TEST

7% Control, Lot # 746.046

1.0 Objective:

To demonstrate that the test product has the antimicrobial properties of the label claim.

2.0 References:

- 2.1 21 CFR 333. Topical antimicrobial drug products for over-the counter human use.
- 2.2 Microconsult, Inc. Test Method MC-14.1 Antiseptic Testing for OTC Drug Products.

3.0 Test Organisms:

Cultures of the following microorganisms are maintained as stock cultures from which working inoculum are prepared. The viable microorganisms used in this test must not be more than four passages removed from the original stock culture. For purposes of the test, one passage is defined as the transfer of organisms from an established culture to fresh medium. All transfers are counted.

- 3.1 Methicillin Resistant *Staphylococcus aureus* (ATCC No. 33591, Quality Technologies, Inc.)
- 3.2 *Clostridium difficile* (ATCC No. 9689, Quality Technologies, Inc.)

4.0 Materials:

- 4.1 Test tubes with closures
- 4.2 Pipettes, 10.0 ml and 1.0 ml serological
- 4.3 Petri dishes, culture loops, and other microbiological apparatus
- 4.4 Anaerobic gas pack

5.0 Media:

- 5.1 Tryptic Soy Agar with lecithin and tween 80
- 5.2 DE Neutralizing Broth

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6.0 Procedure:

6.1 Preparation of Test Samples.

- 6.1.1 Accurately weigh 9.9 gm of product into an appropriately labeled or coded specimen cup.
- 6.1.2 Store test samples at ambient temperature.

6.2 Preparation of Inoculum:

- 6.2.1 Inoculate the surface of a suitable volume of solid agar medium from a recently grown stock culture of each of the specified microorganisms. Incubate the anaerobic bacterial cultures at 35°C +/- 2C for approximately 96 hours under anaerobic conditions.
- 6.2.2 Incubate the aerobic bacterial cultures at 35°C +/- 2C for approximately 24-48 hours under aerobic conditions.
- 6.2.3 To harvest the bacterial culture, place a loop full of the test microorganisms from the plate into tube containing sterile phosphate buffered saline and vortex. Adjust the count with sterile saline or additional microorganisms so that the concentration of the inoculum level is between 10⁻⁷ and 10⁻⁸ microorganisms per milliliter of product.
- 6.2.4 Determine the number of viable microorganisms in each milliliter of the inoculum suspensions by serial dilution in sterile phosphate buffered saline:
- 6.2.5 Plate dilutions of 10⁻⁶, 10⁻⁷, and 10⁻⁸ for the test organism.
- 6.2.6 Overlay with approximately 20 ml of 45°C Tryptic Soy Agar with lecithin and tween 80.
- 6.2.7 Incubate for 96 hours at 35°C +/- 2C for the test organism.
- 6.2.8 Count test organism.
- 6.2.9 Calculate the number of organisms as colony forming units per ml (cfu/ml) of inoculum as follows:

$$\frac{\text{cfu/ml (0.1 ml)}}{9.9 \text{ ml}} = \text{cfu/ml of product}$$

6.3 Inoculation and Plating of Samples

- 6.3.1 Aseptically transfer 0.1 ml of the test suspension into the appropriately labeled 9.9 gm sample of test material. The test organism is inoculated as a pure culture into a single 9.9 gm sample of test material.
- 6.3.2 Thoroughly mix or stir all samples by vortex.
- 6.3.3 Let stand for five seconds and thirty seconds.
- 6.3.4 Remove aliquots at indicated time and transfer to 9.0 ml sterile DE Neutralizing Broth.
- 6.3.5 Perform serial dilutions from 10⁻² to 10⁻⁵.

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- 6.3.6 Transfer 1.0 ml of each dilution into a 100 x 15 mm petri plate in duplicate.
- 6.3.7 Overlay with approximately 20 ml of 45°C Tryptic Soy Agar with lecithin and tween 80.
- 6.3.8 Gently swirl plates and allow to solidify.
- 6.3.9 Incubate aerobic plates for 96 hours at 35°C. under aerobic conditions and anaerobic plates under anaerobic conditions.

6.4 Sample Evaluation

- 6.4.1 Read plates and record results on appropriate data sheet.
- 6.4.2 Using the calculated inoculum concentration of each test microorganism, calculate the log reduction of each microorganism for each kill rate.

6.5 Records and Reports

- 6.5.1 A permanent copy of the recorded data will be maintained by the laboratory for a period of not less than two years
- 6.5.2 A written report will be issued upon completion of the study.

7.0 Data Sheets

7.1 Kill rate Results

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Initiated 11-17-04

Result Date 11-21-04

Organisms	Inoculum Level	10 ⁻²	10 ⁻³	10 ⁻⁵	Average	Log Reduction
S. aureus 5 Seconds	7.33 x10 ⁷	55	9	0	7,175	4.0092820
S. aureus 30 Seconds	7.33 x10 ⁷	62	8	0	1,800	4.6098314
C. difficile 5 Seconds	1.70 x10 ⁷	7	2	0	<1	7.2304489
C. difficile 30 Seconds	1.70 x10 ⁷	15	3	0	<1	7.2304489
		0	0	0		
		0	0	0		
		0	0	0		

Completed by Aym Date 11-21-04

Reviewed by WSD Date 11-23-04