MICROCONSULT, INC.
Microbiological & Analytical Testing Laboratory

Kill Rate Report

4" x 4" Protego Antimicrobial Wound Dressing
Lot: 15187

Initiated: December 31, 2019

Prepared for Global Health Solutions

3218 Commander Drive Suite 100 Carrollton, TX 75006
Phone (972) 250-2902 Fax (972) 250-2903

MICROCONSULT, INC.

Microbiological & Analytical Testing Laboratory

January 22, 2020

Eric Luo
Global Health Solutions
5959 Topanga Blvd. #170
Woodland Hills, CA 91367

Mr. Eric Luo,

On December 31, 2019, Microconsult, Inc. initiated a kill rate test on the following Global Health Solutions sample submission. The 4" x 4" Protego Antimicrobial Wound Dressing, lot 15187 results are listed on the following pages. The product was tested against seven microorganisms; *Escherichia coli* (ATCC No. 8739), *Enterococcus faecalis-VRE* (ATCC No. 51575), *Staphylococcus aureus* (ATCC No. 6538), Methicillin-resistant *Staphylococcus aureus* (MRSA) (ATCC No. 33591), *Pseudomonas aeruginosa* (ATCC No. 9027), *Candida albicans* (ATCC No. 10231), and *Aspergillus brasiliensis* (ATCC No. 16404) at three days, five days, seven days, ten days, and fourteen days. A minimum 2-log reduction is required to claim antimicrobial activity which was achieved by the product at three days against *E. coli, E. faecalis, S. aureus,* MRSA, *P. aeruginosa,* and *C. albicans*. The product also reached a 2-log reduction against *A. brasiliensis* at fourteen days. If you have any questions, please call me at your convenience.

Sincerely,

Alix Paulsen
Microbiology Technician II

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KILL RATE TEST

Objective:

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

References:

A. 21 CFR 333. Topical antimicrobial drug products for over-the counter human use.
B. Microconsult, Inc. Test Method MCP 011_00 Kill Rate Testing.

Test Organisms:

Cultures of the following microorganisms are maintained as stock cultures from which working inocula are prepared. The viable microorganisms used in this test must not be more than five 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.

A. *Escherichia coli* (ATCC No. 8739, Microbiologics)
B. *Enterococcus faecalis*-VRE (ATCC No. 51575, Microbiologics)
C. *Staphylococcus aureus* (ATCC No. 6538, Microbiologics)
D. Methicillin-resistant *Staphylococcus aureus* (MRSA) (ATCC No. 33591, Microbiologics)
E. *Pseudomonas aeruginosa* (ATCC No. 9027, Microbiologics)
F. *Candida albicans* (ATCC No. 10231, Microbiologics)
G. *Aspergillus brasiliensis* (ATCC No. 16404, Microbiologics)

Materials:

A. Test tubes with closures
B. Specimen Cups
C. Pipettes, 10.0 ml and 1.0 ml serological
D. Petri dishes, culture loops, and other microbiological apparatus

Media:

A. Tryptic Soy Agar with Lecithin and Tween 80
B. Sabouraud Dextrose Agar with Chloramphenicol
C. DE Neutralizing Broth
D. Sterile Phosphate Buffered Saline

Procedure:

A. Preparation of Inoculum:
    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 bacterial cultures at 30-35°C for 24-48 hours and the fungal cultures at 20-25°C for 24-48 hours.

2. Determine the number of viable microorganisms in each milliliter of the inoculum suspensions by serial dilution in sterile phosphate buffered saline.
3. Plate dilutions of 10^{-6} and 10^{-7} for the test organisms.
4. Overlay with approximately 20 ml of 45°C Tryptic Soy Agar or 45°C Sabouraud Dextrose Agar as appropriate for the microorganism.
5. Incubate for 24-48 hours at 37°C for bacterial organisms and 3-5 days for the fungal organisms.
6. Count test organisms.
7. Calculate the number of organisms as colony forming units per ml (cfu/ml) of inoculum. Calculate the final concentration in the product as follows:

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

B. Preparation of Test Samples:
   1. Aseptically place one bandage into an appropriately labeled or coded specimen cup as per microorganism and dilution.
   2. Store test samples at 37°C.

C. Inoculation and Plating of Samples:
   1. Aseptically transfer 0.1 ml of the test organism into the appropriately labeled specimen cup with test material. Thoroughly mix all samples.
   2. Allow the samples to stand for the indicated time intervals.
   3. At the indicated times, add contents of a nine ml container of sterile DE neutralizing broth into sample and mix well. Remove 1 ml and transfer to 9 ml of sterile DE neutralizing broth to obtain 10^{-1}, 10^{-3}, and 10^{-5} serial dilutions.
   4. In duplicate, transfer 1.0 ml of each dilution into a 100 x 15 mm petri plate.
   5. Overlay with approximately 20 ml of 45°C Tryptic Soy Agar or 45°C Sabouraud Dextrose Agar as appropriate for the microorganism.
   6. Gently swirl plates and allow to solidify.
   7. Incubate plates for 48-72 hours at 37°C for bacterial organisms and 3-5 days for fungal organisms.

D. Sample Evaluation:
   1. Read plates and record results on appropriate data sheet.
   2. Using the calculated inoculum concentration for each test microorganism, calculate the log reduction for each microorganism to determine kill rate.

E. Records and Reports:

1. The laboratory will maintain a permanent copy of the recorded data for a period of no less than four years.
2. A written report will be issued upon completion of the study.

Data Sheets

1. Kill Rate Results

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Global Health Solutions

4" x 4" Protego Antimicrobial Wound Dressing
Lot: 15187

Initiated: 12/31/19 Results: 01/19/20

Organisms	Inoculum (cfu/ml)	Average cfu/dressing	Log Reduction
E. coli 3 Day	6.92×10^5	No Growth	5.84
E. coli 5 Day	6.92×10^5	No Growth	5.84
E. coli 7 Day	6.92×10^5	No Growth	5.84
E. coli 10 Day	6.92×10^5	No Growth	5.84
E. coli 14 Day	6.92×10^5	No Growth	5.84
E. faecalis 3 Day	7.37×10^5	35	4.32
E. faecalis 5 Day	7.37×10^5	No Growth	5.87
E. faecalis 7 Day	7.37×10^5	No Growth	5.87
E. faecalis 10 Day	7.37×10^5	No Growth	5.87
E. faecalis 14 Day	7.37×10^5	No Growth	5.87
S. aureus 3 Day	6.16×10^5	60	4.01
S. aureus 5 Day	6.16×10^5	No Growth	5.79
S. aureus 7 Day	6.16×10^5	No Growth	5.79
S. aureus 10 Day	6.16×10^5	No Growth	5.79
S. aureus 14 Day	6.16×10^5	No Growth	5.79

Completed by _____ Date 1·22·20

Reviewed by _____ Date 1.23.20

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4" x 4" Protego Antimicrobial Wound Dressing
Lot: 15187

Initiated: 12/31/19 Results: 01/19/20

Organisms	Inoculum (cfu/ml)	Average cfu/dressing	Log Reduction
MRSA 3 Day	6.06 X 10^5	No Growth	5.78
MRSA 5 Day	6.06 X 10^5	No Growth	5.78
MRSA 7 Day	6.06 X 10^5	No Growth	5.78
MRSA 10 Day	6.06 X 10^5	No Growth	5.78
MRSA 14 Day	6.06 X 10^5	No Growth	5.78
P. aeruginosa 3 Day	4.52 X 10^5	No Growth	5.66
P. aeruginosa 5 Day	4.52 X 10^5	No Growth	5.66
P. aeruginosa 7 Day	4.52 X 10^5	No Growth	5.66
P. aeruginosa 10 Day	4.52 X 10^5	No Growth	5.66
P. aeruginosa 14 Day	4.52 X 10^5	No Growth	5.66
C. albicans 3 Day	6.69 X 10^5	3,000	2.35
C. albicans 5 Day	6.69 X 10^5	125	3.73
C. albicans 7 Day	6.69 X 10^5	130	3.71
C. albicans 10 Day	6.69 X 10^5	840	2.90
C. albicans 14 Day	6.69 X 10^5	255	3.42

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Organisms	Inoculum (cfu/ml)	Average cfu/dressing	Log Reduction
A. brasiliensis 3 Day	2.88 X 10^5	23,500	1.09
A. brasiliensis 5 Day	2.88 X 10^5	33,000	0.94
A. brasiliensis 7 Day	2.88 X 10^5	19,000	1.18
A. brasiliensis 10 Day	2.88 X 10^5	5,500	1.72
A. brasiliensis 14 Day	2.88 X 10^5	940	2.49

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