Micro Quality Labs Inc.
3125 N. North Damon Way,
Burbank, California 91505, Phone: 818-845-0070
www.microqualitylabs.com

Customer: Releaf Technologies
Attn: N/A
Address: 11107 Hwy 90 E
Kingsbury, TX 78638

Report Date: 05/08/2023
Date Received: 03/20/2023
Date Completed: 05/05/2023
P.O. #: 1576
Accession #: 39280

SAMPLE DESCRIPTION:

Sample Name: Tetraid Wound Care
Product Code: N/A
Batch/Lot #: 230271

TEST PERFORMED

Kill Time Study

METHOD REFERENCE

ASTM E2315 - 16 Standard Guide for Assessment of Antimicrobial Activity Using a Time-Kill Procedure

PROCEDURE SUMMARY:

The organisms are prepared by inoculating the surface of Soybean–Casein Digest Agar (TSA) plates, incubated at 30 to 35°C for 18 to 24 hours, and Sabouraud Dextrose Agar (SDA) plates, incubated at 20 to 25°C for 3-5 days. Following the incubation period, the plates are washed with sterile Serological Saline Solution to harvest the microorganisms used and dilutions with Saline are made, plated on TSA incubated at 30 to 35°C for 72 hours, and plated on SDA incubated at 20 to 25°C for 3-5 days to determine the concentration. The inoculum level is then adjusted to 108 cfu/mL for use as a stock suspension. Stock suspensions are well mixed and homogenized at each inoculation interval.

The following microorganisms were used in this Kill Time Study to demonstrate the antimicrobial properties of the Tetraid Wound Care against common pathogenic organisms: Microbiologics Kwik-Stiks Staphylococcus aureus ATCC 6538, Escherichia coli ATCC 8739, Pseudomonas aeruginosa ATCC 9027, and Methicillin-Resistant Staphylococcus aureus (MRSA) ATCC 33591.

Positive controls are performed at initiation and completion by pour plating to enumerate inoculum levels and verify culture purity during testing and Negative controls are performed to establish sterility of media, reagents, and materials used at initiation. Neutralizer Suitability using Modified Letheen Broth (MLB) is performed with concurrently with Kill Time testing to confirm the recovery of <100 CFU of the test organism in the subculture media in the presence of product.

Duplicate 10 mL containers for each treated specimen or material concentration is prepared, equilibrated to 25 ± 2 °C, and 0.1 mL of inoculum is added to each container to achieve a final concentration of 10^6 cfu/mL into the product.

Serial dilutions from each replicate are made at intervals of 30 second, 1 minute, and 5 minutes using 1ml of the inoculated test product into 9ml MLB from 1:10 to 1:1000000. Subsequently, 1 mL from each dilution is pour plated with TSA for bacteria, incubated at 30 to 35°C for 72 hours, and SDA for fungus incubated at 20 to 25°C for 5 days, both in duplicate. After the incubation period, all plates are counted to determine the number of microorganisms, results are averaged and reported as $\log_{10}$ reductions.

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NEUTRALIZER SUITABILITY:

Method Suitability Data						
Test Microorganism	**Sample Preparation**	**Inoculum cfu/g***	**Dilution**	**Control**	**Microbial Recovery**	**Percent Recovery**
Staphylococcus aureus ATCC 6538	**NEUTRALIZER SUITABILITY** Sample 1ml of Product Into 9 mL MLB Incubate 20-25°C for 5 min Inoculate 0.1mL organism Plate 1mL onto TSA Incubate 30-35°C 48hrs Plate 1mL onto SDA Incubate 20-25°C 5 days	4.0×10^3	1:100	41cfu/gm	36cfu/gm	87.80%
Escherichia coli ATCC 8739		5.1×10^3	1:100	46cfu/gm	44cfu/gm	95.65%
Pseudomonas aeruginosa ATCC 9027		2.0×10^3	1:100	18cfu/gm	16cfu/gm	88.89%
Methicillin-Resistant Staphylococcus aureus (MRSA) ATCC 33591		2.1×10^3	1:100	24cfu/gm	21cfu/gm	88%

Negative Controls: No Growth **Cfu/gm=colony forming units per gram*

Suitability Test Result: Based on the data observed, recovery of the test organisms confirms the suitability of the test method ASTM E2315 – 16.

MAY 0 8 2023

Reviewed by: Connie Truong Date:
Microbiologist

MAY 0 8 2023

Approved by: Monica Ayala Date:
Microbiologist

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DATA CALCULATION:

Staphylococcus aureus ATCC 6538						
Exposure Time	Concentration of Organism cfu/mL		Percent Reduction		Log_{10} Reduction	
	CONTROL	PRODUCT	CONTROL	PRODUCT	CONTROL	PRODUCT
Initial	$3.0x10^6$	N/A	N/A	N/A	N/A	N/A
Time 30 sec	N/A	<100	N/A	99.99%	N/A	4.477
Time 1 minute	N/A	<100	N/A	99.99%	N/A	4.477
Time 5 minute	N/A	<100	N/A	99.99%	N/A	4.477

Negative Controls: No Growth **Cfu/gm=colony forming units per gram*

Escherichia coli ATCC 8739						
Exposure Time	Concentration of Organism cfu/mL		Percent Reduction		Log_{10} Reduction	
	CONTROL	PRODUCT	CONTROL	PRODUCT	CONTROL	PRODUCT
Initial	$5.0x10^6$	N/A	N/A	N/A	N/A	N/A
Time 30 sec	N/A	<100	N/A	99.99%	N/A	4.700
Time 1 minute	N/A	<100	N/A	99.99%	N/A	4.700
Time 5 minute	N/A	<100	N/A	99.99%	N/A	4.700

Negative Controls: No Growth **Cfu/gm=colony forming units per gram*

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DATA CALCULATION:

Pseudomonas aeruginosa ATCC 9027						
Exposure Time	Concentration of Organism cfu/mL		Percent Reduction		Log_{10} Reduction	
	CONTROL	PRODUCT	CONTROL	PRODUCT	CONTROL	PRODUCT
Initial	$1.0x10^6$	N/A	N/A	N/A	N/A	N/A
Time 30 sec	N/A	<100	N/A	99.99%	N/A	4.000
Time 1 minute	N/A	<100	N/A	99.99%	N/A	4.000
Time 5 minute	N/A	<100	N/A	99.99%	N/A	4.000

Negative Controls: No Growth **Cfu/gm=colony forming units per gram*

Methicillin-Resistant Staphylococcus aureus (MRSA) ATCC 33592						
Exposure Time	Concentration of Organism cfu/mL		Percent Reduction		Log_{10} Reduction	
	CONTROL	PRODUCT	CONTROL	PRODUCT	CONTROL	PRODUCT
Initial	$1.9x10^6$	N/A	N/A	N/A	N/A	N/A
Time 30 sec	N/A	<100	N/A	99.99%	N/A	4.279
Time 1 minute	N/A	<100	N/A	99.99%	N/A	4.279
Time 5 minute	N/A	<100	N/A	99.99%	N/A	4.279

Negative Controls: No Growth **Cfu/gm=colony forming units per gram*

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Relcaf Technologies
1-888-712-1047

Report Date: 05/08/2023
Date Received: 03/20/2023
Date Completed: 05/05/2023
P.O. #: 1576
Accession #: 39280

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Result Calculations:

$$\text{Percent Reduction} = \frac{\text{initial population} - \text{surviving population}}{\text{initial population}} \times 100$$

Log_{10} (initial population) – Log10 (surviving population) = Log_{10} reduction

CONCLUSION:

The project #39280 indicates a 99.99% reduction value at 30 seconds, 1 minute and 5 minute for Staphylococcus aureus ATCC 6538, Escherichia coli ATCC 8739, Pseudomonas aeruginosa ATCC 9027, and Methicillin-Resistant Staphylococcus aureus (MRSA) ATCC 33591 .

The aforementioned results on this report are representative of the samples submitted and may not be indicative of the entire manufacture, batch, and/or lot. Applicable current GMP's shall always be used when sampling. GLP's shall always be practiced by Micro Quality Labs to ensure the most accurate results.

MAY 0 8 2023

Reviewed by: Connie Truong Date:
Microbiologist

MAY 0 8 2023

Approved by: Monica Ayala Date:
Microbiologist