



MICROBIOLOGICAL REPORT

ShowerPill
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Oakland, CA 94612

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TEST PERFORMED

Time Kill/Log Reduction

BTS METHOD #:

M213.R04

The log reduction is used to determine the effectiveness of a product at reducing a specific microorganism population.

Escherichia coli ATCC# 11229, *Staphylococcus aureus* ATCC# 6538, *Klebsiella pneumonia* ATCC# 10031, and *Pseudomonas aeruginosa* ATCC# 9027 were used for this testing.

The microorganism to be tested, 1g of test product, and 1mL of Phosphate Buffer Solution (PBS) was added into separate sterile whirl-pak bags. Each 1g sample of test product and PBS was inoculated with approximately 0.01 mL of the 10⁷-10⁸ CFU/mL suspension. This inoculum resulted in approximately 10⁵ - 10⁶ CFU/mL into the product and PBS control.

At the time intervals of 30 seconds, 1 minute, 5 minutes, 1.0 mL from the inoculated test product was taken and placed into 9.0mL of neutralizing broth (1:10 dilution). Additional 1:10 serial dilutions were prepared using neutralizing broth to achieve 1:100 and 1:1000 dilutions.

One milliliter from each dilution was plated in duplicate and Tryptic Soy Agar with Lecithin & Tween was used as the growth medium. The plates were incubated at 32.5 ± 2.5°C for a minimum of 48 hours. The same procedure was repeated for the D/E Neutralizing Broth control. After the incubation period, all plates were counted to determine the number of microorganisms remaining at each time point.

RESULTS

Escherichia coli ATCC# 11229

EXPOSURE TIME	CONCENTRATION OF ORGANISM (CFU/mL)		%REDUCTION		LOG REDUCTION	
	CONTROL	PRODUCT	CONTROL	PRODUCT	CONTROL	PRODUCT
Initial	2.0E5	2.0E5	N/A	N/A	N/A	N/A
30 sec.	1.5E5	4.4E1	25.0	99.98	0.1	3.7
1 min.	1.5E5	<1.0E1	25.0	>99.99	0.1	4.3
5 min.	1.5E5	<1.0E1	25.0	>99.99	0.1	4.3

Staphylococcus aureus ATCC# 6538

CONCENTRATION OF ORGANISM

<u>EXPOSURE</u> <u>TIME</u>	<u>(CFU/mL)</u>		<u>% REDUCTION</u>		<u>LOG REDUCTION</u>
	<u>CONTROL</u>	<u>PRODUCT</u>	<u>CONTROL</u>	<u>PRODUCT</u>	<u>PRODUCT</u>
Initial	1.0E5	1.0E5	N/A	N/A	N/A
30 sec.	1.8E5	1.5E1	-80.0	99.99	3.8
1 min.	1.6E5	7.5E1	-60.0	99.93	3.1
5 min.	1.4E5	<1.0E1	-40.0	>99.99	4.0

Klebsiella pneumoniae ATCC# 10031

CONCENTRATION OF ORGANISM

<u>EXPOSURE</u> <u>TIME</u>	<u>(CFU/mL)</u>		<u>% REDUCTION</u>		<u>LOG REDUCTION</u>
	<u>CONTROL</u>	<u>PRODUCT</u>	<u>CONTROL</u>	<u>PRODUCT</u>	<u>PRODUCT</u>
Initial	1.9E5	1.9E5	N/A	N/A	N/A
30 sec.	2.5E5	<1.0E1	-31.6	>99.99	4.3
1 min.	2.4E5	<1.0E1	-26.3	>99.99	4.3
5 min.	2.9E5	<1.0E1	-52.6	>99.99	4.3

Pseudomonas aeruginosa ATCC# 9027

CONCENTRATION OF ORGANISM

<u>EXPOSURE</u> <u>TIME</u>	<u>(CFU/mL)</u>		<u>% REDUCTION</u>		<u>LOG REDUCTION</u>
	<u>CONTROL</u>	<u>PRODUCT</u>	<u>CONTROL</u>	<u>PRODUCT</u>	<u>PRODUCT</u>
Initial	1.4E6	1.4E6	N/A	N/A	N/A
30 sec.	1.9E6	<1.0E1	-35.7	>99.99	5.1
1 min.	1.2E6	<1.0E1	14.3	>99.99	5.1
5 min.	1.4E6	<1.0E1	0.0	>99.99	5.1

DATA CALCULATIONS

The concentration of each microorganism for the control and product is listed for each interval. These numbers are expressed in terms of scientific notation. The next heading represents the “Log Reduction” information for each time point. The calculation is used to express the change (reduction or increase) of the microorganism population relative to a starting inoculum.

The Log₁₀ reduction is calculated as follows:

$$\text{Log}_{10}(\text{initial count}) - \text{Log}_{10}(\text{x time interval}) = \text{Log}_{10} \text{ reduction}$$

DISCUSSION:

The minimum bactericidal concentration is defined by the client as 4 log reduction from the initial inoculum¹. The product did achieve more than 4 log reduction at all time intervals for *Klebsiella pneumonia*, and *Pseudomonas aeruginosa*, *Escherichia coli* at 1 minute and 5 minutes, and *Staphylococcus aureus* at 5 minutes only.

Conclusion

The results indicate that solution does have antibacterial activity against *Klebsiella pneumonia*, and *Pseudomonas aeruginosa* at all time intervals, *Escherichia coli* at 1 minute and 5 minutes, and *Staphylococcus aureus* at 5 minutes only.