



Micro Quality Labs

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MICROBIOLOGICAL REPORT

BGP LLC
Attn: Allan Lord
2118 Wilshire Blvd. # 766
Santa Monica, CA 90403

Report Date: 10/17/08
Date Received: 10/13/08
Date Completed: 10/17/08
Project #: 1522

SAMPLE DESCRIPTION:

<u>ACCESSION #</u>	<u>SAMPLE:</u>	<u>LOT #</u>	<u>BATCH#</u>	<u>QTY.</u>
Project # 1522	MN-03-064B	MN-03064B	N/A	N/A

TEST PERFORMED:

**Bacterial Reduction –
Reference AOAC**

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

Pseudomonas acne ATCC 11827 lot 419831 was prepared by inoculating the surface of anaerobic agar. The plates were then placed in an anaerobic chamber and each microorganism was then incubated at 30 to 35°C for 24 to 72 hours. Following the incubation period the plates were washed with sterile Serological Saline Solution to harvest the microorganisms. Using Culti-Loops microorganisms were grown and adjusted to 10^8 (cfu) colony forming units per mL and used as a stock suspension. An additional 1:10 dilution of the stock suspension was made using Serological Saline Solution to achieve a concentration of approximately 10^7 CFU per mL.

For the microorganism to be tested, 20 mL of test product and 20 mL of Serological Saline Solution was added into separate sterile tubes. Each 20mL sample of test product and Serological Saline Solution was inoculated with 0.2 mL of the 10^7 CFU/mL suspensions. These inoculums resulted in approximately 10^6 CFU/mL into the product and Serological Saline Solution control.

At the time intervals of 30 seconds, 60 seconds, 120 seconds, and 1 hour, 1.0 mL from the inoculated test product was taken and placed into 9.0 mL of Modified Lethen Broth (1:10 Dilution). Additional 1:10 serial dilutions were prepared using neutralizing broth to achieve 1:100 and 1:1000 dilutions.

1 mL from each dilution was plated in sterile Petri dishes and melted TSA agar was added as the growth medium for bacterial organisms.

The bacterial plates were incubated at 30 to 35°C for 48-72 hours. The same procedure was repeated for the Serological Saline Solution control. After the incubation period, all plates were counted to determine the number of microorganisms remaining at the various time points.



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RESULTS:

Pseudomonas acne ATCC 11827 lot 419831 Exp. 01/09

EXPOSURE TIME	CONCENTRATION OF ORGANISM (CFU/mL)		% REDUCTION		LOG REDUCTION	
	CONTROL	PRODUCT	CONTROL	PRODUCT	CONTROL	PRODUCT
INITIAL	6.80 x 10 ⁵					
30 sec.	6.80 x 10 ⁵	<10cfu/gm (1:10 dilution)	No change	100.00%	N/A	6.0
1 min.	6.80 x 10 ⁵	<10cfu/gm (1:10 dilution)	No change	100.00%	N/A	6.0
2 min.	6.80 x 10 ⁵	<10cfu/gm (1:10 dilution)	No change	100.00%	N/A	6.0
1 hour	6.00 x 10 ⁵	<10cfu/gm (1:10 dilution)	12%	100.00%	0.05	6.0

Data Calculation:

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 two headings represent the “% reduction” and “Log Reduction” information for each time point. Both calculations are used to express the change (reduction or increase) of the microorganism population relative to starting inoculums.

$$\% \text{ Reduction} = \frac{\text{Initial Count} - \text{Count at x time interval}}{\text{Initial Count}} \times 100$$

$$\frac{6.8 \times 10^5 - 6.0 \times 10^5}{6.8 \times 10^5}$$

The log reduction is calculated as follows:

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

$$\text{For example: } \text{Log } 10(6.8 \times 10^5) - \text{Log } 10(6.0 \times 10^5) = 5.83 - 5.78 = 0.05 \text{ log reduction}$$

Discussion:

The minimum bactericidal concentration is defined as 99.9% decrease (3 log) in the initial inoculums. The test product had no counts for growth when exposed to *Pseudomonas acne* at all time interval.

Conclusion:

The results indicate that project # 1522 has antimicrobial activity against *Pseudomonas acne*. Culti-Loop, ATCC 11827 at 30 second contact time. 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.

Approved: Karine Aylozyan, Senior Microbiologist / Q.A Coordinator

 10/17/08

Germina Ratquaz, Senior Microbiologist

 10/17/08



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Project # 1522	MN-03-064B	MN-03064B	N/A	N/A

TEST PERFORMED:

**Bacterial Reduction –
Reference AOAC**

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

Methicillin Resistant Staphylococcus aureus (MRSA) was prepared by inoculating the surface of TSA slants. Each microorganism was then incubated at 30 to 35°C for 18 to 24 hours. Following the incubation period the slants were washed with sterile Serological Saline Solution to harvest the microorganisms. Using Culti-Loops microorganisms were grown and adjusted to 10^8 (cfu) colony forming units per mL and used as a stock suspension. An additional 1:10 dilution of the stock suspension was made using Serological Saline Solution to achieve a concentration of approximately 10^7 CFU per mL.

For the microorganism to be tested, 20 mL of test product and 20 mL of Serological Saline Solution was added into separate sterile tubes. Each 20mL sample of test product and Serological Saline Solution was inoculated with 0.2 mL of the 10^7 CFU/mL suspensions. These inoculums resulted in approximately 10^6 CFU/mL into the product and Serological Saline Solution control.

At the time intervals of 30 seconds, 60 seconds, 120 seconds, 180 seconds, and 1 hour, 1.0 mL from the inoculated test product was taken and placed into 9.0 mL of Modified Lethen Broth (1:10 Dilution). Additional 1:10 serial dilutions were prepared using neutralizing broth to achieve 1:100 and 1:1000 dilutions.

1 mL from each dilution was plated in sterile Petri dishes and melted TSA agar was added as the growth medium for bacterial organisms.

The bacterial plates were incubated at 30 to 35°C for 48 hours. The same procedure was repeated for the Serological Saline Solution control. After the incubation period, all plates were counted to determine the number of microorganisms remaining at the various time points.



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Project # 1522

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RESULTS:

Methicillin Resistant Staphylococcus aureus (MRSA)

ATCC 33591 lot # 496453 exp. 01/10

EXPOSURE TIME	CONCENTRATION OF ORGANISM (CFU/mL)		% REDUCTION		LOG REDUCTION	
	CONTROL	PRODUCT	CONTROL	PRODUCT	CONTROL	PRODUCT
INITIAL	3.4×10^5					
30 sec.	3.4×10^5	<10cfu/gm (1:10 dilution)	No change	100.00%	N/A	6.0
1 min.	3.4×10^5	<10cfu/gm (1:10 dilution)	No change	100.00%	N/A	6.0
2 min.	3.4×10^5	<10cfu/gm (1:10 dilution)	No change	100.00%	N/A	6.0
1 hour	3.0×10^5	<10cfu/gm (1:10 dilution)	12%	100.00%	0.05	6.0

Data Calculation:

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 two headings represent the “% reduction” and “Log Reduction” information for each time point. Both calculations are used to express the change (reduction or increase) of the microorganism population relative to starting inoculums.

$$\% \text{ Reduction} = \frac{\text{Initial Count} - \text{Count at x time interval}}{\text{Initial Count}} \times 100$$

For example: % Reduction for *MRSA* at 30 seconds for test product

$$\frac{3.4 \times 10^5 - 3.0 \times 10^5}{3.4 \times 10^5}$$

The log reduction is calculated as follows:

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

$$\text{For example: } \text{Log}_{10}(3.4 \times 10^5) - \text{Log}_{10}(3.0 \times 10^5) = 5.53 - 5.48 = 0.05 \text{ log reduction}$$

Discussion:

The minimum bactericidal concentration is defined as 99.9% decrease (3 log) in the initial inoculums. The test product had no counts for growth when exposed to *Methicillin Resistant Staphylococcus aureus (MRSA)* at all time interval.

Conclusion:

The results indicate that Project # 1522 has antimicrobial activity against *Methicillin Resistant Staphylococcus aureus (MRSA)*, Culti-Loop, ATCC # 33591 at 30 second contact time. 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.

Approved: Karine Aylozvan, Senior Microbiologist / Q.A Coordinator

10/17/08

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