



MICROBIOLOGICAL REPORT

All Terrain
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REVISION #2: 11/09/07
Report Date: 10/31/07
Date Received: 10/24/07
Date Completed: 11/08/07
Project #: 559691
P.O. #: Not Specified
Reference #: 5224-093;
097; 101

SAMPLE DESCRIPTION:

<u>ACCESSION #</u>	<u>SAMPLE:</u>	<u>LOT #</u>	<u>BATCH #</u>
559691	Hand Sanz (TM) Hand Sanitizer	12C87	Not Specified

<u>TEST PERFORMED:</u>	<u>BTS METHOD #:</u>
Log Reduction	M213.R03

SAMPLE PREPARATION:

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

The organisms were prepared by inoculating the surface of tryptic soy agar slants. The microorganism was then incubated at 35.2 ± 2.5°C for 24 hours. Following the incubation period the slants were washed with sterile Phosphate Buffered Saline (PBS) to harvest the microorganisms. The microbial suspension was adjusted to approximately 10⁸ colony forming units (CFU) per mL and labeled as the stock suspension. An additional 1:10 dilution of the stock suspension was made using PBS to achieve a concentration of approximately 10⁷ CFU per mL.

At the time intervals of 30 seconds, 1 and 5 minutes, 1 gram of the inoculated test product was taken and placed into 9.0 mL 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 duplicated and melted tryptic soy agar with lecithin and tween 80 was added as the growth medium for the bacterial organisms. The plates were incubated at 35.2 ± 2.5°C for minimum of 48 hours. The same procedure was repeated for the Phosphate Buffer Saline control. After the incubation period, all plates were counted to determine the number of microorganisms remaining at each time point.

RESULTS:

Staphylococcus aureus ATCC# 6538

<u>EXPOSURE TIME</u>	<u>CONCENTRATION OF ORGANISM (CFU/mL)</u>		<u>% REDUCTION</u>		<u>LOG REDUCTION</u>	
	<u>CONTROL</u>	<u>PRODUCT</u>	<u>CONTROL</u>	<u>PRODUCT</u>	<u>CONTROL</u>	<u>PRODUCT</u>
Initial	2.7E+05	2.7E+05	N/A	N/A	N/A	N/A
30 sec	2.8E+05	<10	-3.7	100.0	0.0	4.4
1 min	2.8E+05	<10	-3.7	100.0	0.0	4.4
5 min	2.9E+05	<10	-7.4	100.0	0.0	4.4

Klebsiella pneumoniae ATCC# 10031

EXPOSURE TIME	CONCENTRATION OF ORGANISM (CFU/mL)		% REDUCTION		LOG REDUCTION	
	CONTROL	PRODUCT	CONTROL	PRODUCT	CONTROL	PRODUCT
Initial	2.5E+05	2.5E+05	N/A	N/A	N/A	N/A
30 sec	2.5E+05	<10	0.0	100.0	0.0	4.4
1 min	2.3E+05	<10	8.0	100.0	0.0	4.4
5 min	2.4E+05	<10	4.0	100.0	0.0	4.4

Pseudomonas aeruginosa ATCC# 9027

EXPOSURE TIME	CONCENTRATION OF ORGANISM (CFU/mL)		% REDUCTION		LOG REDUCTION	
	CONTROL	PRODUCT	CONTROL	PRODUCT	CONTROL	PRODUCT
Initial	2.1E+05	2.1E+05	N/A	N/A	N/A	N/A
30 sec	2.2E+05	<10	-4.8	100.0	0.0	4.3
1 min	2.4E+05	<10	-14.3	100.0	-0.1	4.3
5 min	2.3E+05	<10	-9.5	100.0	0.0	4.3

Escherichia coli ATCC# 11229

EXPOSURE TIME	CONCENTRATION OF ORGANISM (CFU/mL)		% REDUCTION		LOG REDUCTION	
	CONTROL	PRODUCT	CONTROL	PRODUCT	CONTROL	PRODUCT
Initial	3.6E+05	3.6E+05	N/A	N/A	N/A	N/A
30 sec	3.0E+05	<10	16.7	100.0	0.1	4.6
1 min	4.2E+05	<10	-16.7	100.0	-0.1	4.6
5 min	4.8E+05	<10	-33.3	100.0	-0.1	4.6

Staphylococcus aureus (MRSA) ATCC# 33592

EXPOSURE TIME	CONCENTRATION OF ORGANISM (CFU/mL)		% REDUCTION		LOG REDUCTION	
	CONTROL	PRODUCT	CONTROL	PRODUCT	CONTROL	PRODUCT
Initial	2.5E+05	2.5E+05	N/A	N/A	N/A	N/A
15 sec	2.6E+05	<10	-4.0	100.0	0.0	4.4
30 sec	2.7E+05	<10	-8.0	100.0	0.0	4.4
1 min	2.5E+05	<10	0.0	100.0	0.0	4.4
5 min	2.8E+05	<10	-12.0	100.0	0.0	4.4

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 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 as 3 log reduction from the initial inoculum¹. The product achieved more than 3 log reduction at all time intervals against all organisms.

Conclusion:

The results indicate that the Hand Sanz (TM) Hand Sanitizer have antibacterial activity against *S. aureus*, *K. pneumoniae*, *P. aeruginosa*, *E. coli*, and *S. aureus* (MRSA).



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¹ Disinfection, Sterilization, and Preservation Fourth Edition, Seymour S. Block, pg. 1035