MICROCHEM LABORATORY

STUDY REPORT

Study Title

Antibacterial Activity and Efficacy of Non-porous Test Substances from Silver Defender

Test Method

Japanese Industrial Standard Z 2801
Antibacterial Products – Test for Antibacterial Activity and Efficacy

Study Identification Number

NG14123

Study Sponsor

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(201) 921-2632
alan@silverdefender.com

Test Facility

Microchem Laboratory
1304 W. Industrial Blvd
Round Rock, TX 78681
(512) 310-8378
Testing conducted by: Chris Craney

JIS Z 2801: General Information

The Japanese Industrial Standard Committee (JIS) is an international organization that develops and standardizes test methods for a variety of products and materials. The JIS method Z 2801 is a quantitative test designed to assess the performance of antimicrobial finishes on hard, non-porous surfaces. The method can be conducted using contact times ranging from ten minutes up to 24 hours. For a JIS Z 2801 test, non-antimicrobial control surfaces are used as the baseline for calculations of microbial reduction. The method is versatile and can be used to determine the antimicrobial activity of a diverse array of surfaces including plastics, metals, and ceramics.

Laboratory Qualifications Specific to JIS Z 2801

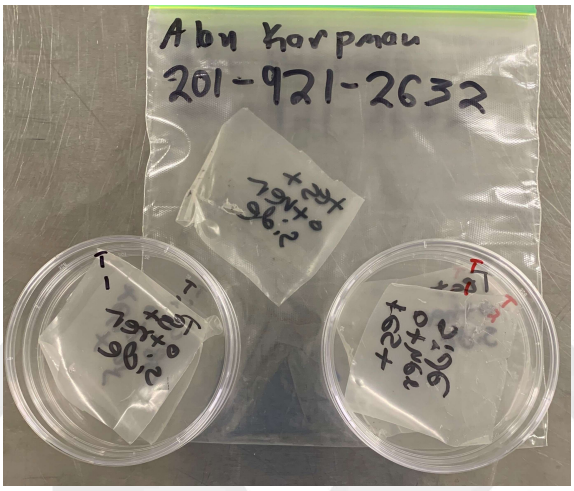
Microchem Laboratory began conducting the JIS Z 2801 test method in 2007. Since then, the laboratory has performed thousands of JIS Z 2801 tests on a broad array of test substances, against myriad bacteria, fungi, and viruses. The laboratory is skilled with regard to modifications of the method to accommodate customer needs. Every JIS Z 2801 test at Microchem Laboratory is performed in a manner that is appropriate for the test substances submitted by the Study Sponsor, while maintaining the integrity of the study.

Study Timeline

Cultures Initiated	Surfaces Inoculated	Surfaces Incubated	Surfaces Harvested	Surfaces Evaluated	Report Delivered
04NOV2019	05NOV2019	05NOV2019	05NOV2019 06NOV2019	09NOV2019 11NOV2019	21NOV2019

Test Substance Information

Test substances were received on 04 NOV 2019.



The photo above shows the samples received for testing and the placement of test samples in petri dishes prior to inoculation. The surface opposite the side labeled "test other side" was tested in this study.

Test Substances Received and used in testing: Silver Defender Clinical Sample

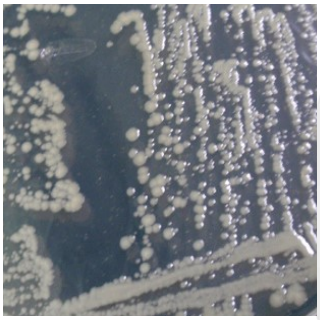
Test Substances arrived in dimensions that were optimal for the conduct of the Study. Test substances did not need to be cut down to ideal sizes for the Study.

Test Microorganism Information

The test microorganisms selected for this test:

Staphylococcus aureus 6538

This bacterium is a Gram-positive, spherical-shaped, facultative anaerobe. *Staphylococcus* species are known to demonstrate resistance to antibiotics such as methicillin. *S. aureus* pathogenicity can range from commensal skin colonization to more severe diseases such as pneumonia and toxic shock syndrome (TSS). *S. aureus* is commonly used in several test methods as a model for gram positive bacteria. It can be difficult to disinfect but does demonstrate susceptibility to low level disinfectants.



Escherichia coli 8739

This bacteria is a Gram-negative, rod shaped, facultative anaerobe commonly found in the gastrointestinal tract of mammals. Although most serotypes of this microorganism are harmless there are pathogenic groups of *E. coli* such as enterohemorrhagic (EHEC), verocytotoxin producing (VTEC) and Shiga-like toxin producing (STEC) that can cause a multitude of illnesses. *E. coli* is relatively susceptible to disinfection when dried on a surface, yet it can be a challenging microorganism to mitigate in solution.

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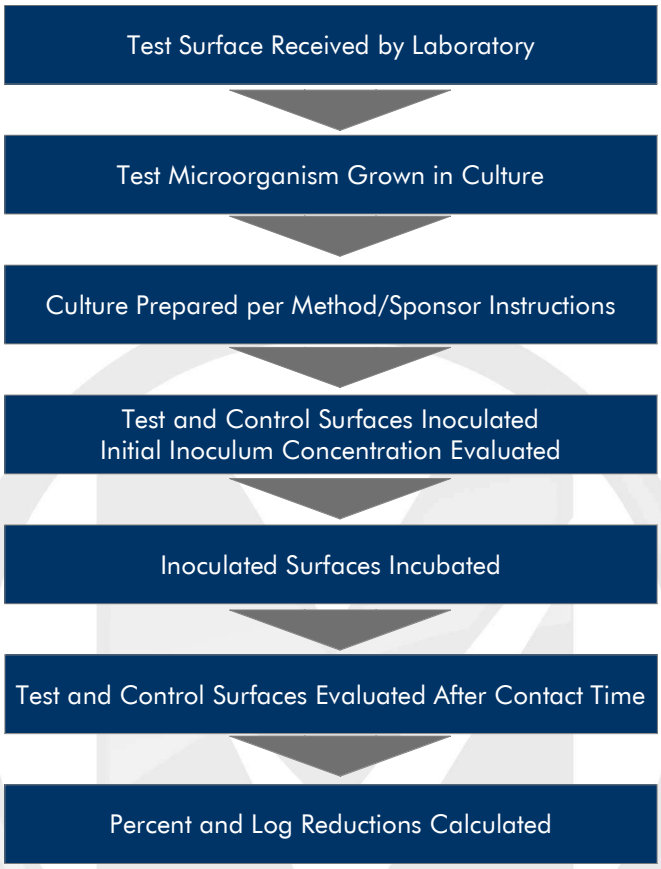
Diagram of the Procedure



Summary of the Procedure

- The test microorganism is prepared, usually by growth in a liquid culture medium.
- The suspension of test microorganism is standardized by dilution in a nutritive broth (this affords microorganisms the opportunity to proliferate during the test).
- Control and test surfaces are inoculated with microorganisms, and then the microbial inoculum is covered with a thin, sterile film. Covering the inoculum spreads it, prevents it from evaporating, and ensures close contact with the antimicrobial surface.
- Microbial concentrations are determined at "time zero" by elution followed by dilution and plating to agar.
- A control is run to verify that the neutralization/elution method effectively neutralizes the antimicrobial agent in the antimicrobial surface being tested.
- Inoculated, covered control and antimicrobial test surfaces are allowed to incubate undisturbed in a humid environment for 24 hours, usually at body temperature.
- After incubation, microbial concentrations are determined. Reduction of microorganisms relative to the control surface is calculated.

Criteria for Scientific Defensibility of a JIS Z 2801 Study

For Microchem Laboratory to consider a JIS Z 2801 study to be scientifically defensible, the following criteria must be met:

1. The average number of viable bacteria recovered from the time zero samples must be approximately 1 x 10^4 cells/cm^2 or greater.
2. Ordinary consistency between replicates must be observed for the time zero samples.
3. The number of viable bacteria recovered from the control surface after the contact time must not be significantly (>2-Log_{10}) less than the original inoculum concentration.
4. Positive/Growth controls must demonstrate growth of appropriate test microorganism.
5. Negative/Purity controls must demonstrate no growth of test microorganism.

Passing Criteria

JIS specifies a performance criteria for antimicrobial efficacy of greater than or equal to a 2 Log_{10} or 99% reduction in in the test microorganisms when comparing the treated surface to the control surface after the contact time. Alternatively, passing criteria may be determined by the Study Sponsor in accordance with pertinent governmental regulations.

Testing Parameters

Test Substance Size:	50 mm x 50 mm	Film Used? (Size):	Yes (40 mm x 40 mm)
Replicates:	One		

Culture Growth Media:	Tryptic Soy Broth	Culture Growth Time:	18-30 hours
Culture Dilution Media:	See Study Notes	Culture Dilution Supplement:	N/A
Inoculum Concentration:	~2 x 10^5 CFU/Carrier	Inoculum Volume:	0.400 ml
Contact Time:	24 hours ± 1 hour	Contact Temp.:	36°C ± 1°C
Neutralizer:	D/E Broth (10.0 ml)	Enumeration Plate Media:	Tryptic Soy Agar
Enumeration Plate Incubation Temperature:	36°C ± 1°C	Enumeration Plate Incubation Time:	24-30 hours

PARAMETERS

Study Modifications

No study modifications were made for this study.

Study Notes

The culture diluent used in this study was 1:500 Nutrient Broth in Phosphate Buffered Saline.

Study Photographs

The photo above shows the harvest of S. aureus from the surface of sthe Silver Defender Clinical Sample with Dey-Engley neutralizer broth after the 24 hour contact time.

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Control Results

Neutralization Method:	Dey-Engley Broth	Media Sterility:	Sterile
Growth Confirmation:	Confirmed		

Calculations

$$\text{Percent Reduction} = \left(\frac{B - A}{B}\right) \times 100$$

Where:
B = Number of viable test microorganisms on the control carriers after the contact time
A = Number of viable test microorganisms on the test carriers after the contact time

$$\text{Log}_{10}\text{Reduction} = \text{Log}\left(\frac{B}{A}\right)$$

Where:
B = Number of viable test microorganisms on the control carriers after the contact time
A = Number of viable test microorganisms on the test carriers after the contact time

Results for *S. aureus* ATCC 6538

Test Microorganism	Carrier		CFU/Carrier	Average CFU/Carrier	Percent Reduction vs. Microchem Control	Log_{10} Reduction vs. Microchem Control
S. aureus ATCC 6538	Microchem Control		2.65E+05	1.93E+05	N/A	
			1.20E+05			
	Microchem Control		2.80E+05	3.43E+05	N/A	
			4.05E+05			
	Silver Defender Clinical Sample		9.00E+01	N/A	99.97%	3.58
			1.00E+03		99.71%	2.535

Results for *E.coli* ATCC 8739

Test Microorganism	Carrier	Replicate	CFU/Carrier	Average CFU/Carrier	Percent Reduction vs. Microchem Control	Log_{10} Reduction vs. Microchem Control
E. coli ATCC 8739	Microchem Control	1	4.15E+05	4.10E+05	N/A	
		2	4.05E+05			
	Microchem Control	1	9.70E+06	1.23E+07	N/A	
		2	1.49E+07			
	Silver Defender Clinical Sample	1	8.00E+01	N/A	99.9993%	5.19
		2	5.00E+00		99.99996%	6.39

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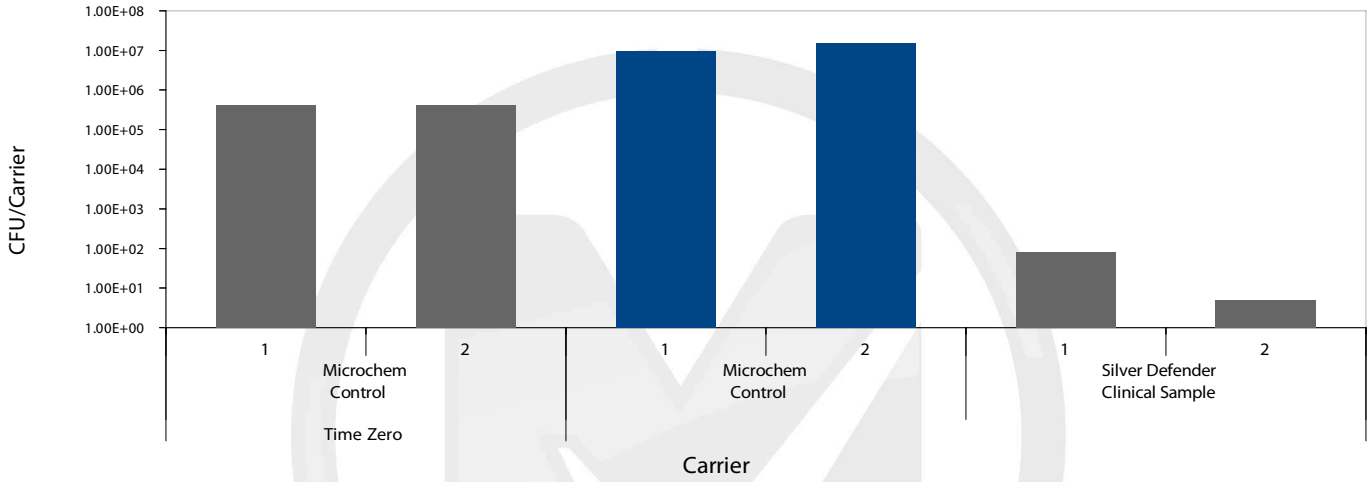

The results of this study apply to the tested substances(s) only. Extrapolation of findings to related materials is the responsibility of the Sponsor.