MICROCHEM LABORATORY

STUDY REPORT

Study Title

Antibacterial Efficacy of Bio-Care Technology's Non-porous Test Substance

Test Method

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

Study Identification Number

NG7610

Study Sponsor

Bio-Care Technology, LLC

Test Facility

Microchem Laboratory
1304 W. Industrial Blvd
Round Rock, TX 78681
(512) 310-8378

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

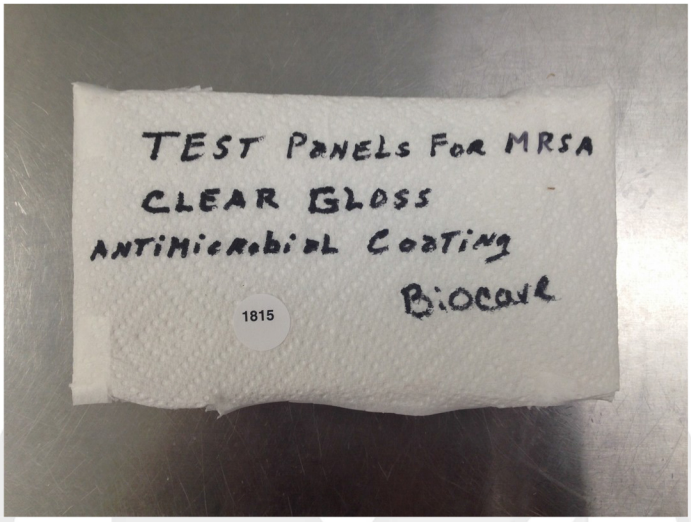
Microchem Laboratory began conducting the JIS Z 2801 test method in 2007. Since then, the laboratory has performed thousands of JIS Z 2801 tests on a broad array of test substances, against myriad bacteria, fungi, and viruses. The laboratory is skilled with regard to modifications of the method to accommodate 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

Culture Initiated	Surfaces Inoculated	Surfaces Incubated	Surfaces Harvested	Surfaces Evaluated	Report Delivered
28AUG2016	29AUG2016	29AUG2016	30AUG2016	01SEP2016	19SEP2016

Test Substance Information

The test substances were received on 23 AUG 2016 and the following picture was taken.

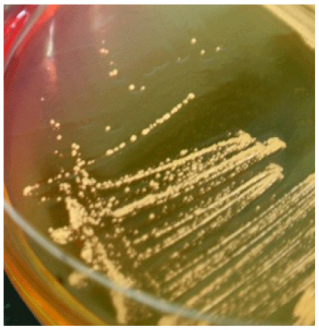


Test Substance Received: Clear Gloss Test Panel (B)
Antimicrobial Coating Test Panel (A)

The test substance arrived in dimensions that were not optimal for the conduct of the study. The test substance was cut down to an ideal size for the Study.

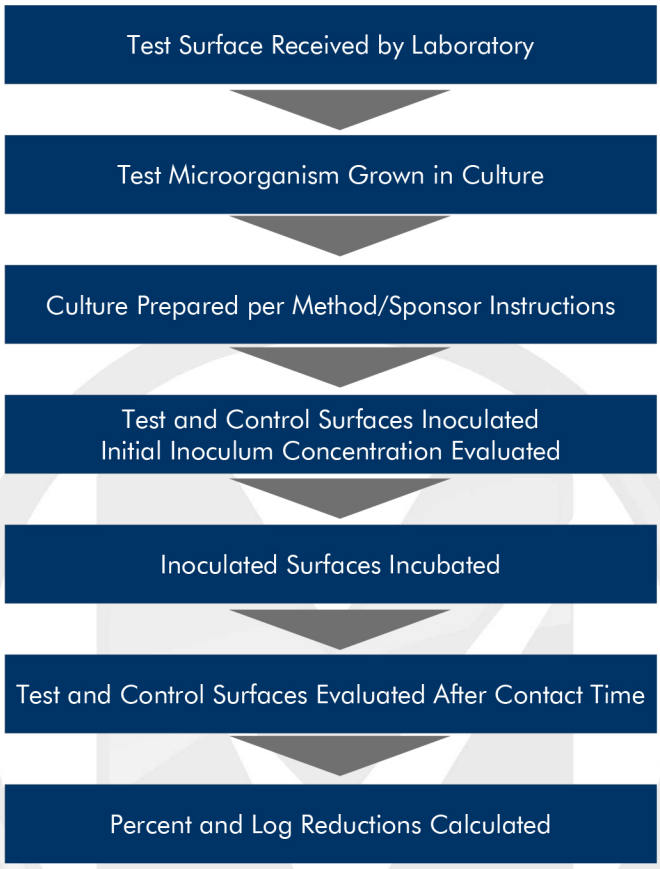
Test Microorganism Information

The test microorganism(s) selected for this test:



Staphylococcus aureus **(MRSA) 33592**
This bacteria is a Gram-positive, cocci shaped, aerobe which is resistant to the penicillin-derivative antibiotic methicillin. MRSA can cause troublesome infections, and their rapid reproduction and resistance to antibiotics makes them more difficult to treat. MRSA bacteria are resistant to drying and can therefore survive on surfaces and fabrics for an extended period of time and therefore makes this bacteria an excellent representative for antimicrobial efficacy testing on surfaces.

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 government regulations.

Testing Parameters used in this Study

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 hours
Culture Dilution Media:	1:500 Nutrient Broth	Culture Dilution Supplement:	N/A
Inoculum Concentration:	~1 x 10^5 CFU/Carrier	Inoculum Volume:	0.400 ml
Contact Time:	24 hr	Contact Temp.:	Ambient
Neutralizer:	D/E Broth (10 ml)	Enumeration Plate Media:	Tryptic Soy Agar
Enumeration Plate Incubation Temperature:	36°C ± 1°C	Enumeration Plate Incubation Time:	24 ± 6 hours

PARAMETERS

Study Modifications

No additional study modification were done.

Study Notes

No additional study notes were taken.

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Control Results

Neutralization Method: N/A
Growth Confirmation: Viable & Pure

Media Sterility: Sterile

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

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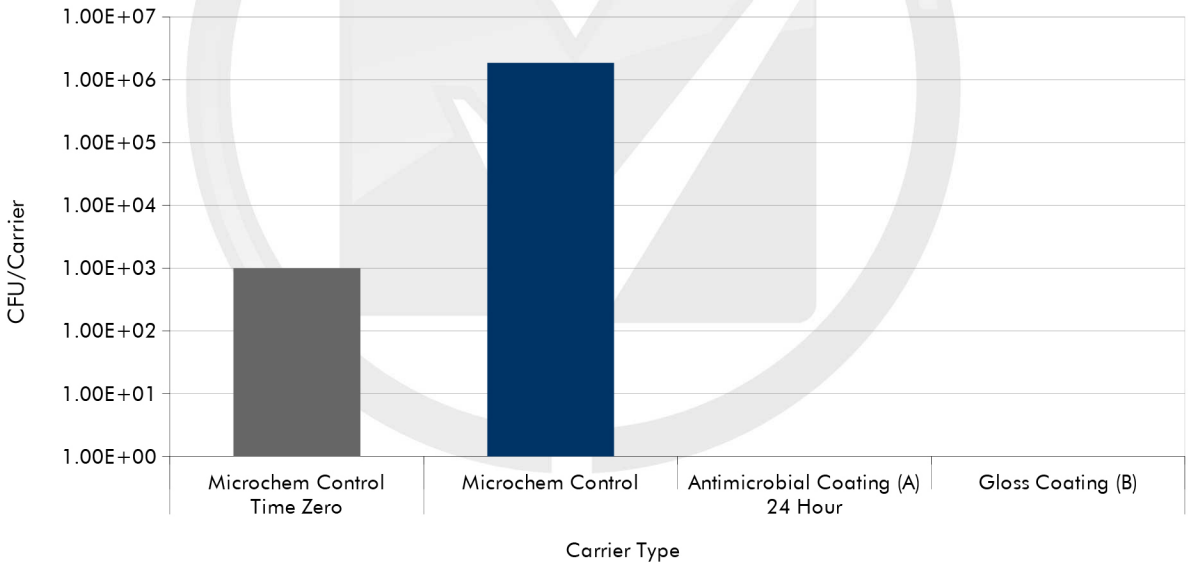
Results of the Study

Test Microorganism	Contact Time	Carrier Type	CFU/Carrier	Percent Reduction Compared to Control at Contact Time	Log_{10} Reduction Compared to Control at Contact Time
S. aureus 6538	Time Zero	Microchem Control	<1.00E+03	N/A	
	24 Hour	Microchem Control	1.85E+06	N/A	
		Antimicrobial Coating (A)	<5.00E+00	>99.9997%	>5.57
		Gloss Coating (B)	<5.00E+00	>99.9997%	>5.57



Note: The limit of detection for this study is 5.00E+00 CFU/Carrier. Values below the limit of detection are shown as <5.00E+00 on the table and zero on the graph.

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