MICROCHEM LABORATORY

STUDY REPORT

Study Title

Antibacterial Activity and Efficacy of Bio-Care Non-porous Test Substance

Test Method

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

Study Identification Number

NG8187

Study Sponsor

Bio-Care

Test Facility

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

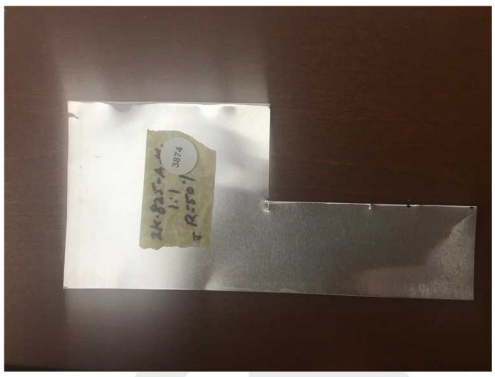
Study Timeline

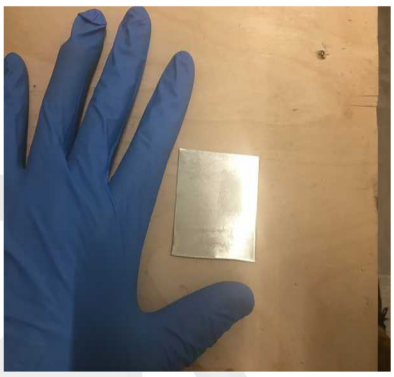
Culture Initiated	Surfaces Inoculated	Surfaces Incubated	Surfaces Harvested	Enumeration Plates Evaluated	Report Delivered
08JAN2017	09JAN2017	09JAN2017	11JAN2017	13JAN2017	21FEB2017

Amended report delivered on 06 MAR 2017.

Test Substance Information

The test substance received on 18NOV2016. The test substance arrived in dimensions that were not optimal for the conduct of the Study. Test substance was cut down to 50mm x 50mm.





(Note: the photos above depict the test surfaces evaluated in this study)

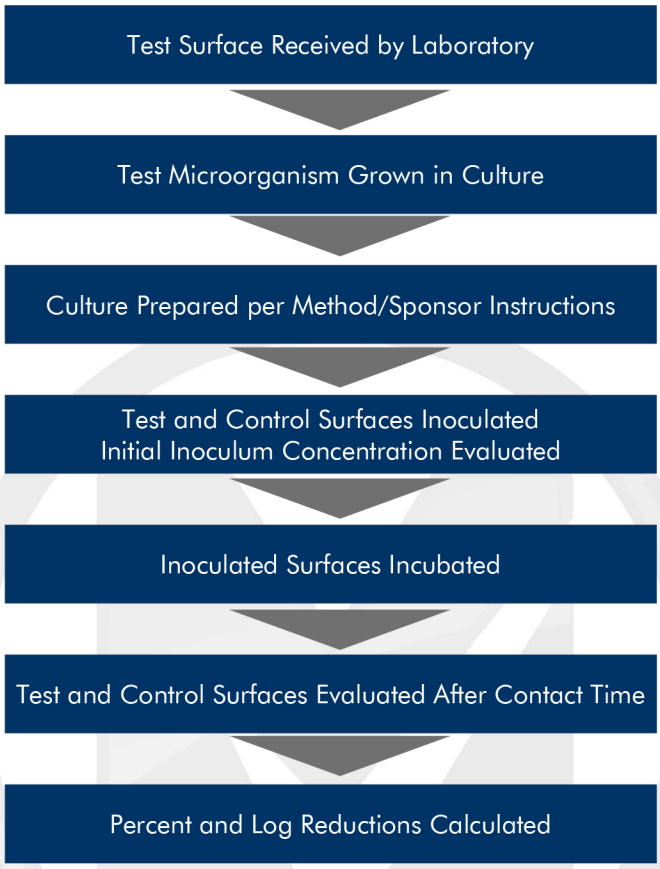
Samples received: 2K-825-A.M. 1:1 J R=50%

Test Microorganism Information

The test microorganism selected for this test:

Staphylococcus aureus **ATCC 33592 (MRSA)**
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 governmental 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		
Inoculum Concentration:	~2.5 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 mL)	Enumeration Plate Media:	Tryptic Soy Agar
Enumeration Plate Incubation Temperature:	36°C ± 1°C	Enumeration Plate Incubation Time:	24 -48 hours

PARAMETERS

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Study Modifications

The Study Material (2K-825-A.M. 1:1 J R=50%) was aged 42 days at 70-80°C prior to test initiation. Daily data entries of the minimum and maximum temperatures for each 24 hour period between thermometer readings of the oven used for the aging process were recorded.

Sample 2K-825-A.M. 1:1 J R=50% was successfully, artificially aged a minimum of 3 years per the parameters of the study.

Study Notes

The Study Material 2K-825-A.M. 1:1 J R=50% was cut down to 50mm x 50mm test sizes after the aging process was concluded.

Report was amended on 06 MAR 2017 to include the time of aging in the results table.

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Control Results

Neutralization Method: N/A Media Sterility: Confirmed
Growth Confirmation: Confirmed – Target Microorganism

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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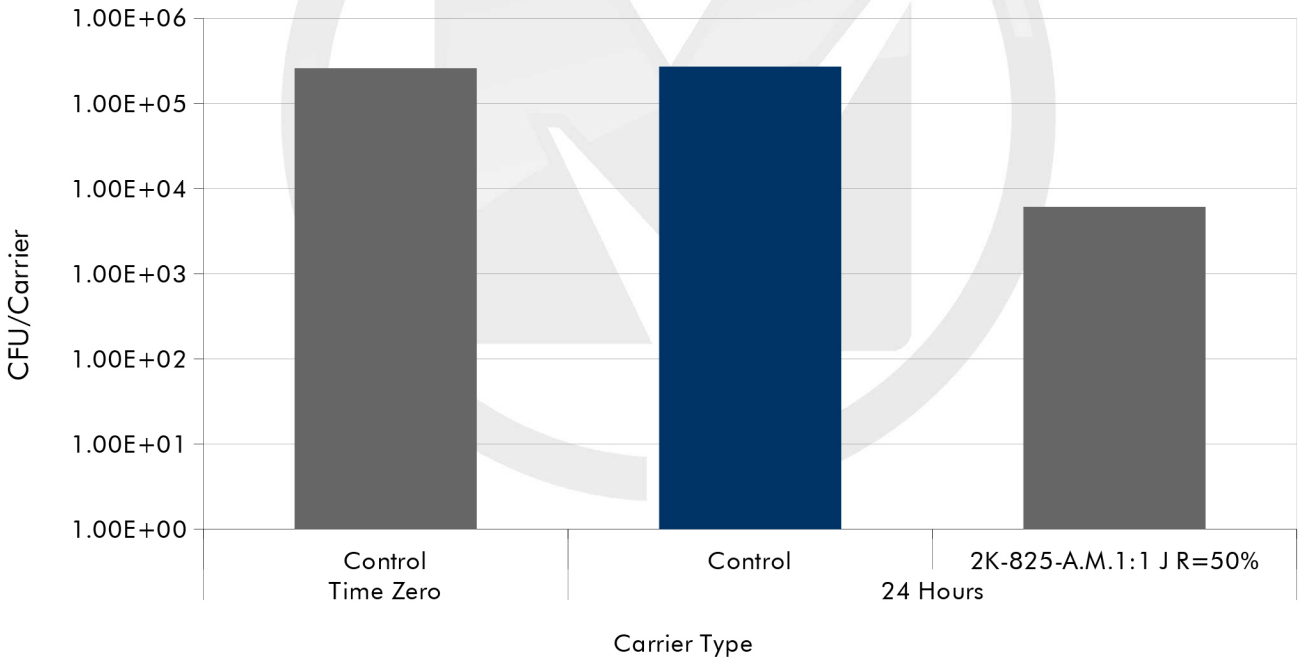
Results of the Study

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 (MRSA) 33592	Time Zero	Control	2.60E+05	N/A	
	24 Hours	Control	2.71E+05		
		2K-825-A.M.1:1 J R=50% (3 year artificial, accelerated aging)	6.10E+03	97.74%	1.65



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