MICROCHEM LABORATORY

STUDY REPORT

Study Title

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

Test Method

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

Study Identification Number

NG13695

Study Sponsor

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Test Facility

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Testing performed by: B. Richard, B.S.

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

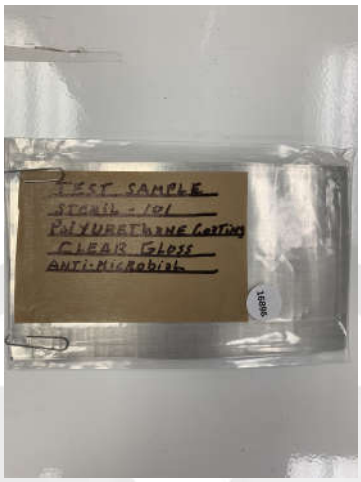
Microchem Laboratory began conducting the JIS Z 2801 test method in 2007. Since then, the laboratory has performed thousands of JIS Z 2801 tests on a broad array of test substances, against myriad bacteria, fungi, and viruses. The laboratory is skilled with regard to modifications of the method to accommodate 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
04NOV2019	07NOV2019	07NOV2019	08NOV2019	12NOV2019	13NOV2019

Test Substance Information

The test substance was received on August 27, 2019 and the following pictures were taken.



Test Substances Received: Steril-101, Polyurethane Coating, Clear Gloss Antimicrobial

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

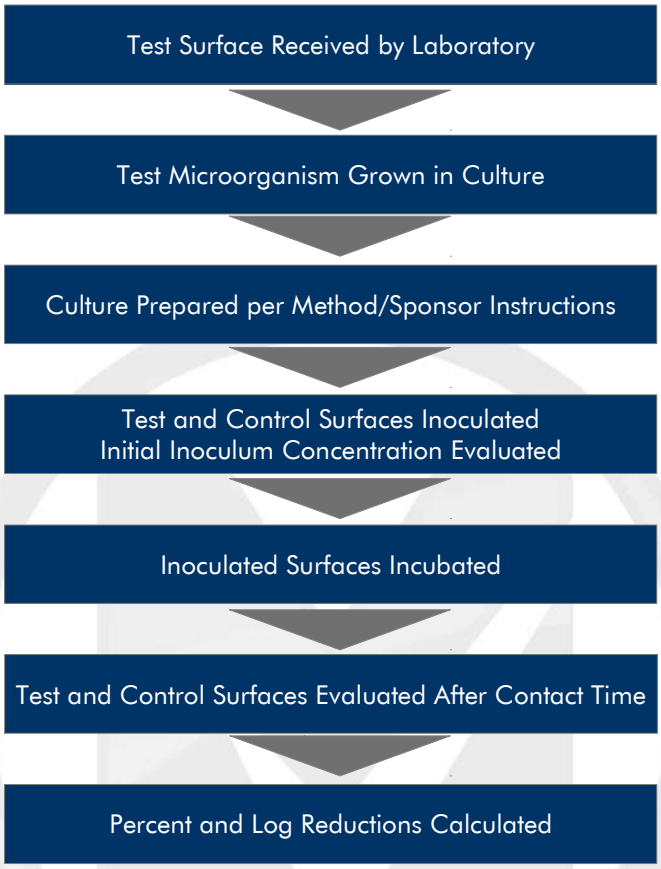
Test Microorganism Information

The test microorganism(s) selected for this test:

Candida auris AR Bank #0381

This fungi grows as a yeast and is ascomycetous. *C. auris* is an emerging pathogen and the epidemiology for transmission is still under investigation. Infections have most often occured in hospitalized patients and healthcare facilities. This yeast has developed resistance to commonly used antifungal drugs and specialized laboratory methods are needed to accurately identify *C. auris* infections. Because of this, *C. auris* infections are increasingly difficult to identify and treat.

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.

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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:	Single		

Culture Growth Media:	SDA	Culture Growth Time:	3-5 days
Culture Dilution Media:	1:500 Nutrient Broth	Culture Dilution Supplement:	N/A
Inoculum Concentration:	8.15 x 10^5 CFU/Carrier	Inoculum Volume:	0.400 mL
Contact Time:	24 hours	Contact Temp.:	36°C ± 1°C
Neutralizer:	D/E Broth (10 mL)	Enumeration Plate Media:	SDA
Enumeration Plate Incubation Temperature:	30°C ± 2°C	Enumeration Plate Incubation Time:	3-5 days

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Study Modifications

The study was modified from the method to use a Study Sponsor requested microorganism. The incubation conditions for the enumeration plates and the incubation for the culture was modified to ensure sufficient growth of the test microorganism.

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Control Results

Neutralization Method: Not evaluated
Growth Confirmation: Growth, pure target
Media Sterility: Confirmed 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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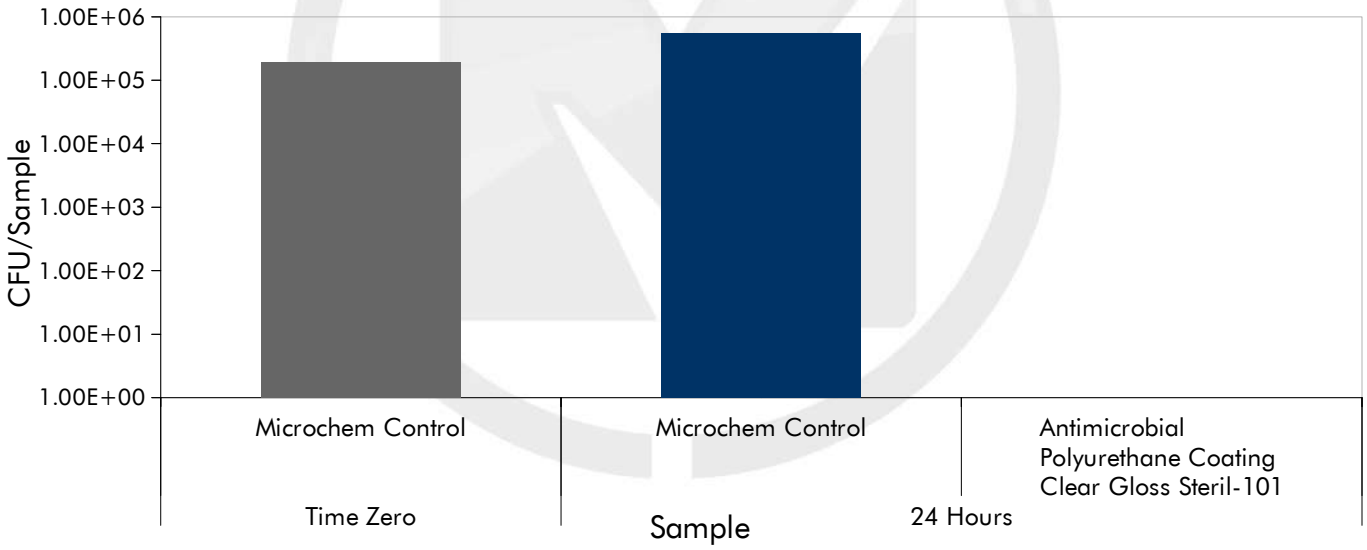
Results of the Study

Test Microorganism	Contact Time	Sample	CFU/Sample	Average CFU/Sample	Percent Reduction vs. Microchem Control at 24 hours	Log_{10} Reduction vs. Microchem Control at 24 hours
C. auris AR Bank #0381	Time Zero	Microchem Control	1.95E+05	1.95E+05	N/A	
	24 Hours	Microchem Control	5.45E+05	5.45E+05	N/A	
		Antimicrobial Polyurethane Coating Clear Gloss Steril-101	<5.00E+00	<5.00E+00	>99.9991%	>5.04



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