MICROCHEM LABORATORY

STUDY REPORT

Study Title

Antibacterial Activity and Efficacy of Bio-Care Technologies USA's Non-porous Test Substances

Test Method

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

Study Identification Number

NG11650

Study Sponsor

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Test Facility

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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

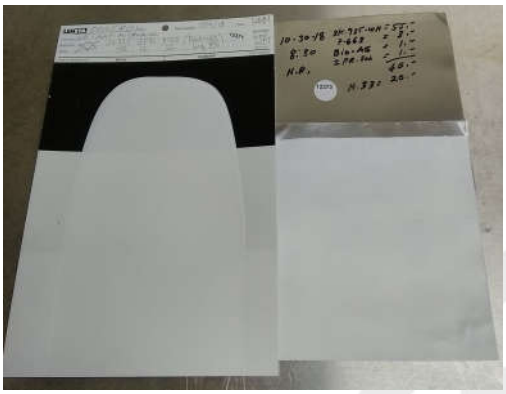
Microchem Laboratory began conducting the JIS Z 2801 test method in 2007. Since then, the laboratory has performed thousands of JIS Z 2801 tests on a broad array of test substances, against myriad bacteria, fungi, and viruses. The laboratory is skilled with regard to modifications of the method to accommodate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

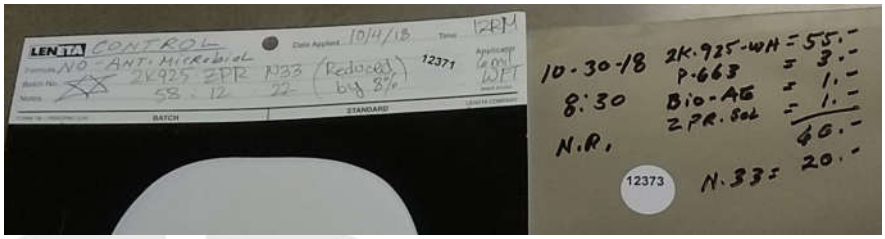
Study Timeline

Culture Initiated	Surfaces Inoculated	Surfaces Incubated	Surfaces Harvested	Surfaces Evaluated	Report Delivered
21 NOV 2018	11 DEC 2018	11 DEC 2018	12 DEC 2018	14 DEC 2018	17 DEC 2018

Test Substance Information

The test substances were received on 06 NOV 2018. The following pictures of samples used in testing was taken.





The photo above is a closeup of sample labels.

Test Substance Received:

(left in photos) Control, No Antimicrobial
Formula: No Antimicrobial
Batch No: 2K925 ZPR N33
Notes: 58 12 22, Reduced by 8%

(right in photos) 2K-925-WH
Formula: P-663
Batch No: Bio-AM-AG
Notes: ZPR-Sol

Test Substance arrived in dimensions that were not optimal for the conduct of the Study. Test substances were cut down for the conduct of the study.

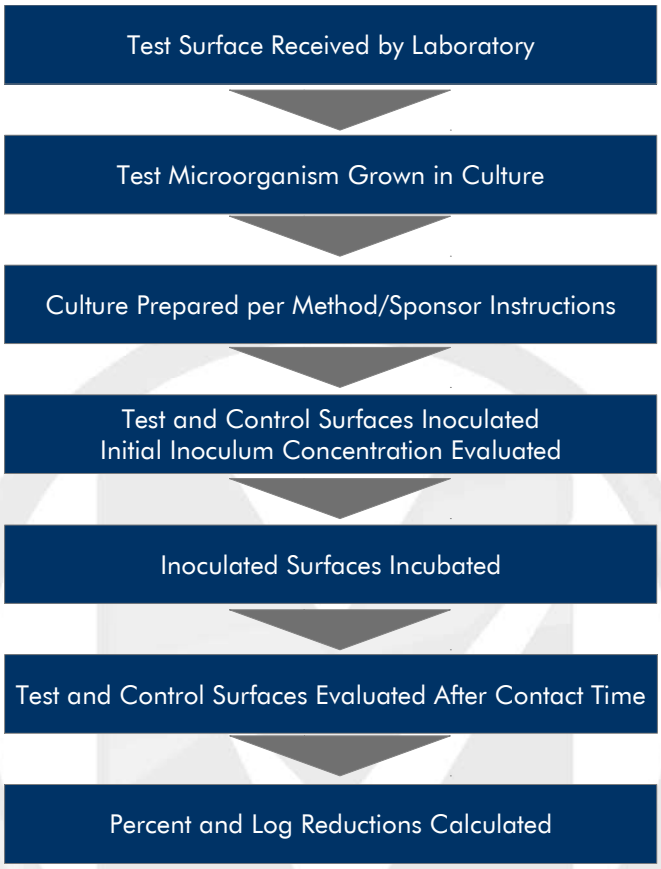
Test Microorganism Information

The test microorganism selected for this test:

***Aspergillus brasiliensis* 9642**

This fungi is a conidiophore, or a sexual spore generating aerobic fungus. *A. brasiliensis*, formerly listed as a strain of *A. niger*, is related to other *Aspergillus* species in that they produce spores which are highly resistant to chemical and environmental conditions. *A. brasiliensis* is commonly used as a benchmark fungus for antimicrobial fungicides and preservatives used in pharmaceutical and personal care products.

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 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:	Single		

Culture Growth Media:	PDA	Culture Growth Time:	See Study Notes
Culture Dilution Media:	1:500 Nutrient Broth	Culture Dilution Supplement:	0.01% Triton X 100
Inoculum Concentration:	~1 x 10^5 CFU/Sample	Inoculum Volume:	0.400 mL
Contact Time:	24 Hours	Contact Temp.:	30°C ± 2°C
Neutralizer:	D/E Broth (10 mL)	Enumeration Plate Media:	PDA
Enumeration Plate Incubation Temperature:	30°C ± 2°C	Enumeration Plate Incubation Time:	18-48 hours

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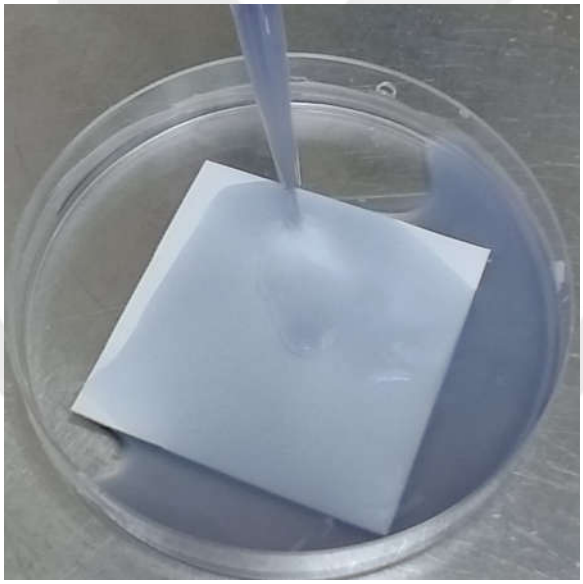
Study Modifications

No modifications were made for the study.

Study Notes

A. brasiliensis (formerly *A. niger*) ATCC 9642 culture was initiated 21 NOV 2018, harvested into Mineral Salt solution on 30 NOV 2018 and stored at 5°C±5°C until use. Culture incubated at 30°C ± 2°C for 9 days.

Study Photos



The photograph above shows the harvesting of A.niger 9642 (A. brasiliensis) from the surface of the test sample at the 24 hour contact time.

Control Results

Neutralization Method: Dey Engley Broth
Growth Confirmation: Confirmed
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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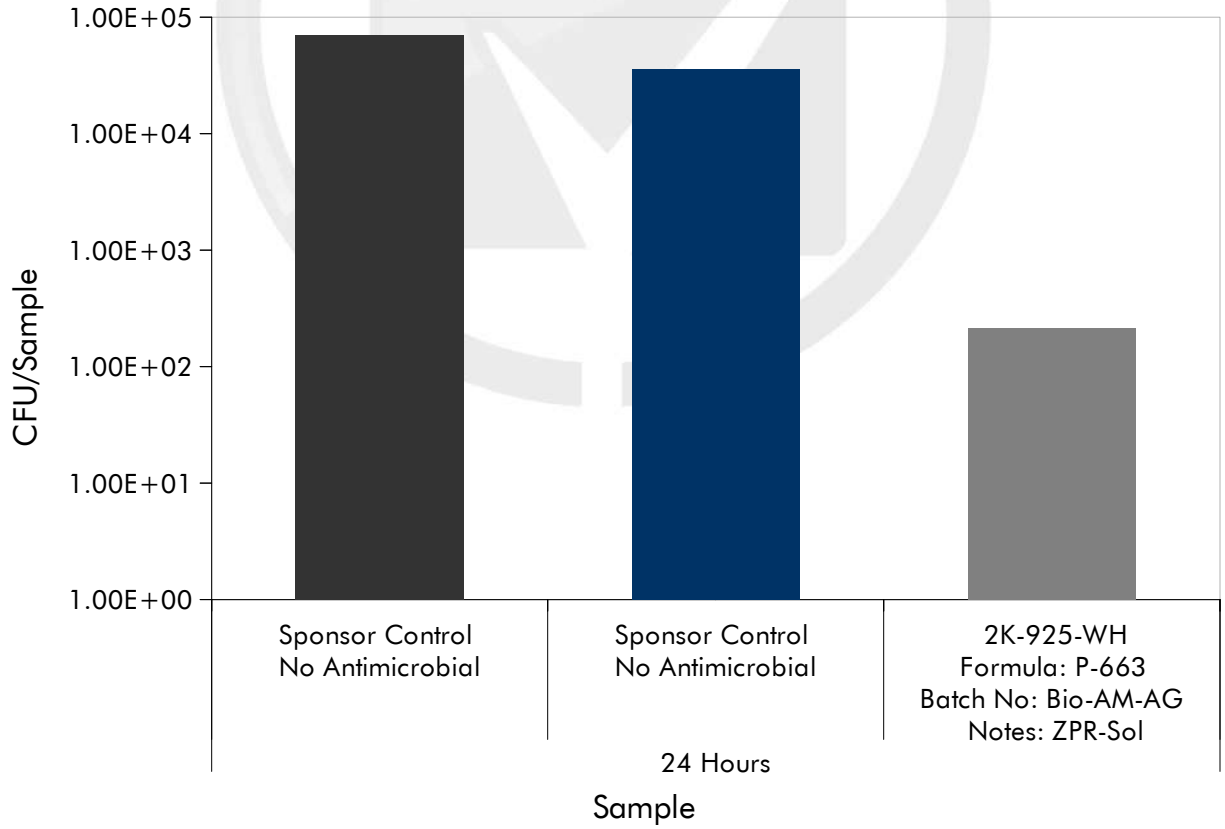
Results of testing with *A.niger* 9642 *(A. brasiliensis)*

Test Microorganism	Contact Time	Sample	Replicate	CFU/Sample	Percent Reduction vs. Control at 24 hours	Log_{10} Reduction vs. Control at 24 hours
A. brasiliensis 9642	Time Zero	Sponsor Control No Antimicrobial	1	7.00E+04	N/A	
	24 Hour	Sponsor Control No Antimicrobial	1	3.60E+04	N/A	
		2K-925-WH Formula: P-663 Batch No: Bio-AM-AG Notes: ZPR-Sol	1	2.15E+02	99.40%	2.22



RESULTS

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

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