MICROCHEM LABORATORY

STUDY REPORT

Study Title

Antibacterial Activity and Efficacy of Non-porous Test Substance from XPEL, Inc.

Test Method

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

Study Identification Number

NG13980-V1

Study Sponsor

Abhishek Joshi
XPEL, Inc.
618 W Sunset Rd.
San Antonio, TX 78216
(240) 839-2387
ajoshi@xpel.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

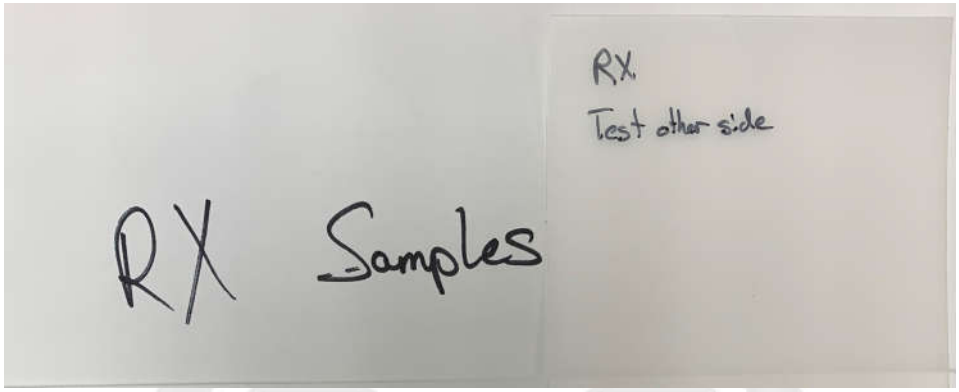
Microchem Laboratory began conducting the JIS Z 2801 test method in 2007. Since then, the laboratory has performed thousands of JIS Z 2801 tests on a broad array of test substances, against myriad bacteria, fungi, and viruses. The laboratory is skilled with regard to modifications of the method to accommodate 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
21OCT2019	22OCT2019	22OCT2019	22OCT2019 23OCT2019	24OCT2019 25OCT2019	01NOV2019

Test Substance Information

The test substance was received on 15 OCT 2019 and the following picture was taken.



Test substance received: RX sample, adhesive on film's treated surface.
Label directions were followed, the liner opposite the active side was removed in order to test the active side surface.

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

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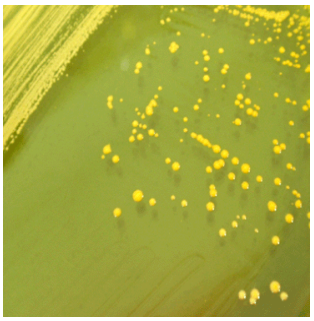
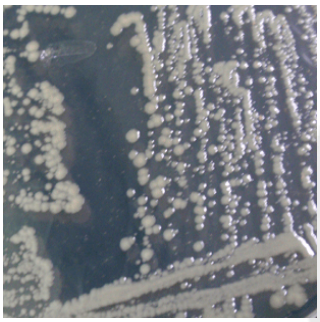
Test Microorganism Information

The test microorganism(s) 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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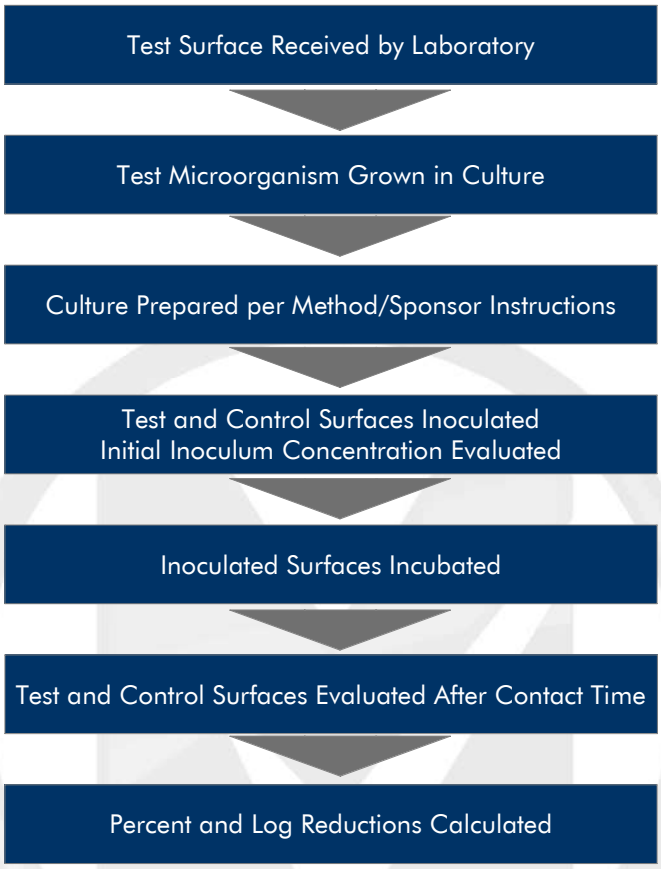
Diagram of the Procedure



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:	1:500 Nutrient Broth	Culture Dilution Supplement:	N/A
Inoculum Concentration:	~2 x 10^5 CFU/Sample	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:	18-48 hours

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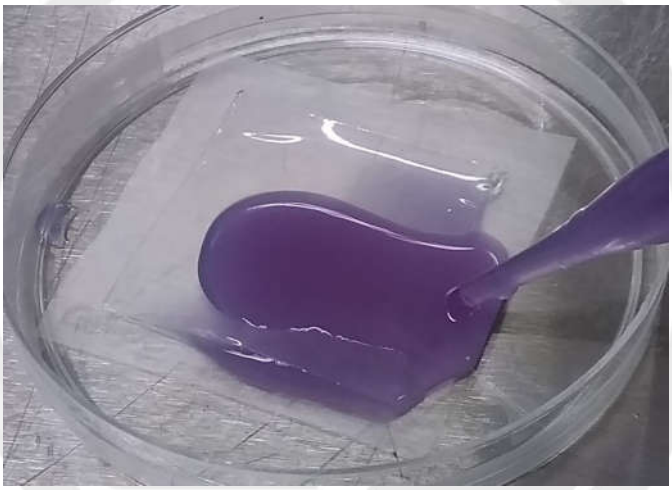
Study Modifications

No further modifications were made to the method for this study.

Study Notes

No additional notes were made for this study.

Study Photographs



The photo above shows the harvesting of E. coli ATCC 8739, with Dey-Engley broth, from the RX sample, replicate 2, at 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

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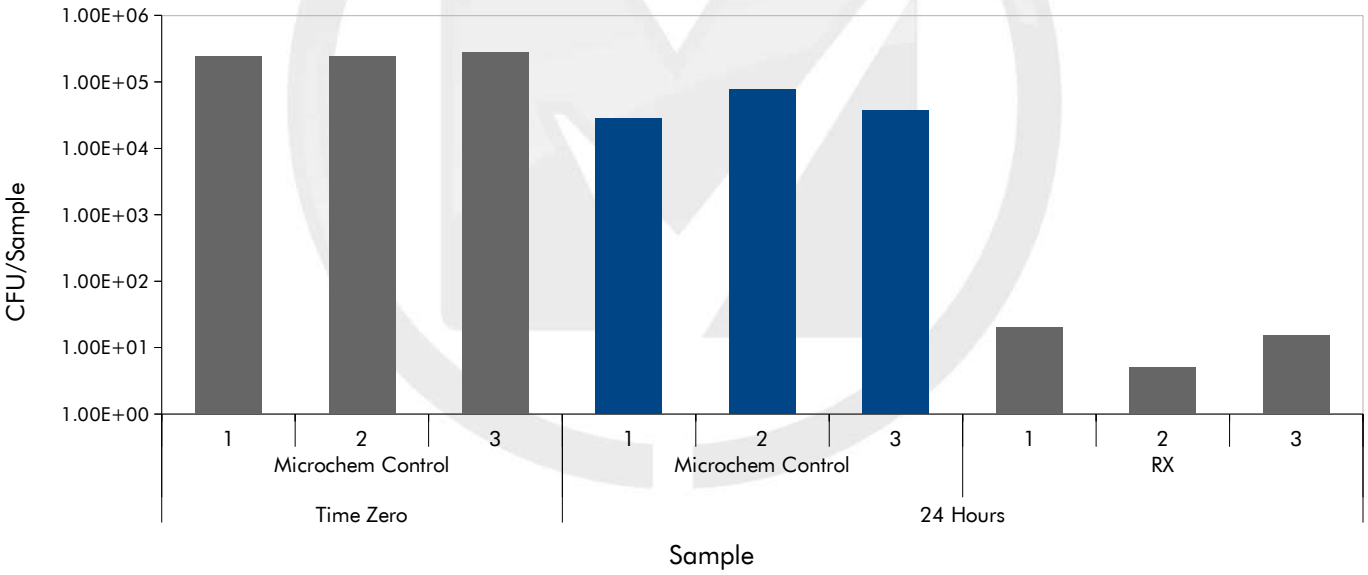
Results of the Study- *S. aureus* ATCC 6538

Test Microorganism	Contact Time	Sample	Replicate	CFU/Sample	Average CFU/Sample	Percent Reduction vs. Microchem Control at 24 hours	Log_{10} Reduction vs. Microchem Control at 24 hours
S. aureus ATCC 6538	Time Zero	Microchem Control	1	2.40E+05	2.55E+05	N/A	
			2	2.45E+05			
			3	2.80E+05			
	24 Hours	Microchem Control	1	2.85E+04	4.78E+04	N/A	
			2	7.75E+04			
			3	3.75E+04			
		RX	1	2.00E+01	1.33E+01	99.97%	3.55
			2	5.00E+00			
			3	1.50E+01			



RESULTS

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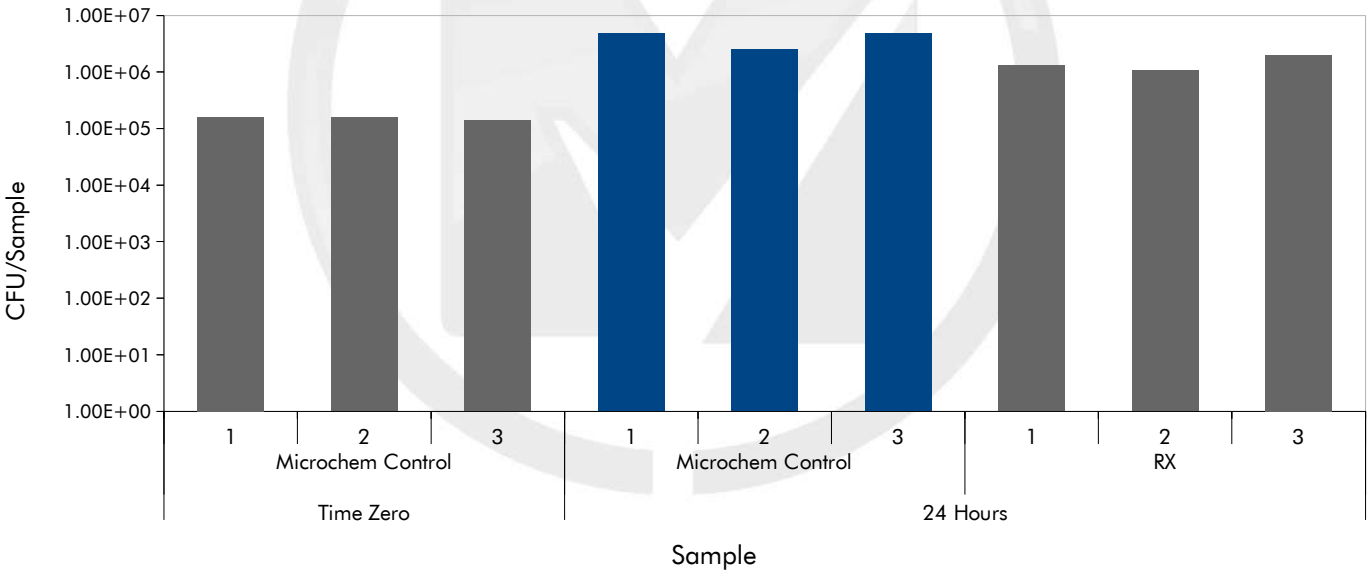
Results of the Study- *E. coli* ATCC 8739

Test Microorganism	Contact Time	Sample	Replicate	CFU/Sample	Average CFU/Sample	Percent Reduction vs. Microchem Control at 24 hours	Log_{10} Reduction vs. Microchem Control at 24 hours
E. coli ATCC 8739	Time Zero	Microchem Control	1	1.60E+05	1.52E+05	N/A	
			2	1.55E+05			
			3	1.40E+05			
	24 Hours	Microchem Control	1	4.85E+06	4.02E+06	N/A	
			2	2.50E+06			
			3	4.70E+06			
		RX	1	1.30E+06	1.45E+06	63.90%	0.44
			2	1.05E+06			
			3	2.00E+06			



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