MICROCHEM LABORATORY

STUDY REPORT

Study Title

Antibacterial Activity and Efficacy of ClearSteam Technologies' Treated Test Surfaces

Test Method

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

Study Identification Number

NG12398-4A

Study Sponsor

Jim Praechtl
ClearStream Technologies
801 Clanton Road
Suite C-104
Charlotte, NC 28217

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

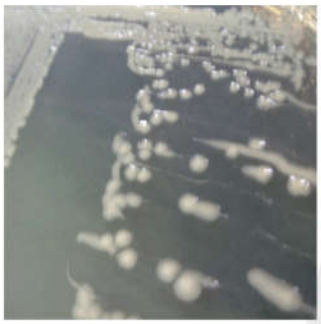
Culture Initiated	Surfaces Inoculated	Surfaces Incubated	Surfaces Harvested	Surfaces Evaluated	Report Delivered
13MAY2019	14MAY2019	14MAY2019	14MAY2019 15MAY2019	16MAY2019	10JUN2019

Test Substance Information

The test substances were received on 04MAR2019.
Test substances were received ready to use for testing.

Test Microorganism Information

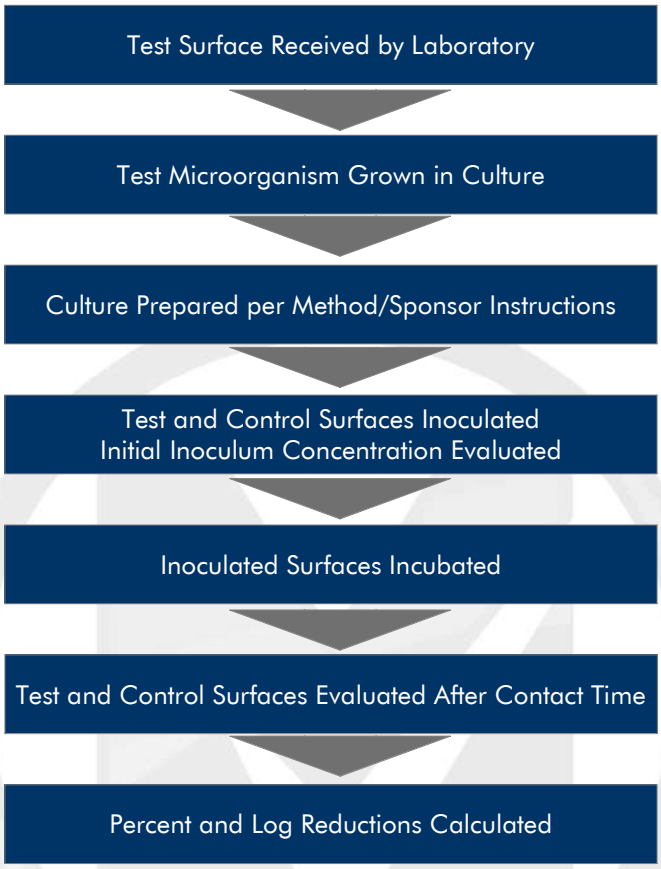
The test microorganism(s) selected for this test:



Salmonella enterica 10708
This bacteria is Gram-negative, rod-shaped, facultative anaerobe. Like the closely related *Escherichia* genus, *Salmonella* are common to all parts of the world and share habitats in the digestive systems of cold and warm-blooded animals. *S. enterica* is one of the most common bacteria associated with zoonotic and foodbourne illness. Because of it's regular occurrence and pathogenicity, *S. enterica* is a common bacteria for measuring disinfectant efficacy.

1304 W. INDUSTRIAL BLVD. ROUND ROCK, TX 78681 | © 2016 | CONTACT@MICROCHEMLAB.COM | 512.310.8378

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.
- 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.

1304 W. INDUSTRIAL BLVD. ROUND ROCK, TX 78681 | © 2016 | CONTACT@MICROCHEMLAB.COM | 512.310.8378

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 10^5 cells/carrier 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 Surface Type	Acrylic	Test Surface Size	50 mm x 50 mm
Cover Film Used?	Yes	Cover Film Size	40 mm x 40 mm
Replicates	2	Culture Growth Media:	Tryptic Soy Broth
Culture Dilution Media:	1:500 Nutrient Broth	Culture Growth Time:	~48 hours
Inoculum Concentration:	≥1.0 x 10^6 CFU/ml	Culture Dilution Supplement:	N/A
Contact Time:	24 hours	Inoculum Volume:	0.400 ml
Neutralizer:	D/E Broth (10 ml)	Contact Temp.:	36 ± 1°C
Enumeration Plate Incubation Temperature:	36°C ± 1°C	Enumeration Plate Media:	Tryptic Soy Agar
Enumeration Plate Incubation Time:	~48 hours		

Study Modifications

Due to labeling error by Study Sponsor the test and control carriers were reversed while the test was being performed. This error was discovered following review of the results and confirmed with carriers not used in testing. The names of the carriers have been changed within this report such that they reflect the appropriate carrier type.

1304 W. INDUSTRIAL BLVD. ROUND ROCK, TX 78681 | © 2016 | CONTACT@MICROCHEMLAB.COM | 512.310.8378

Control Results

Growth Confirmation: Positive, 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

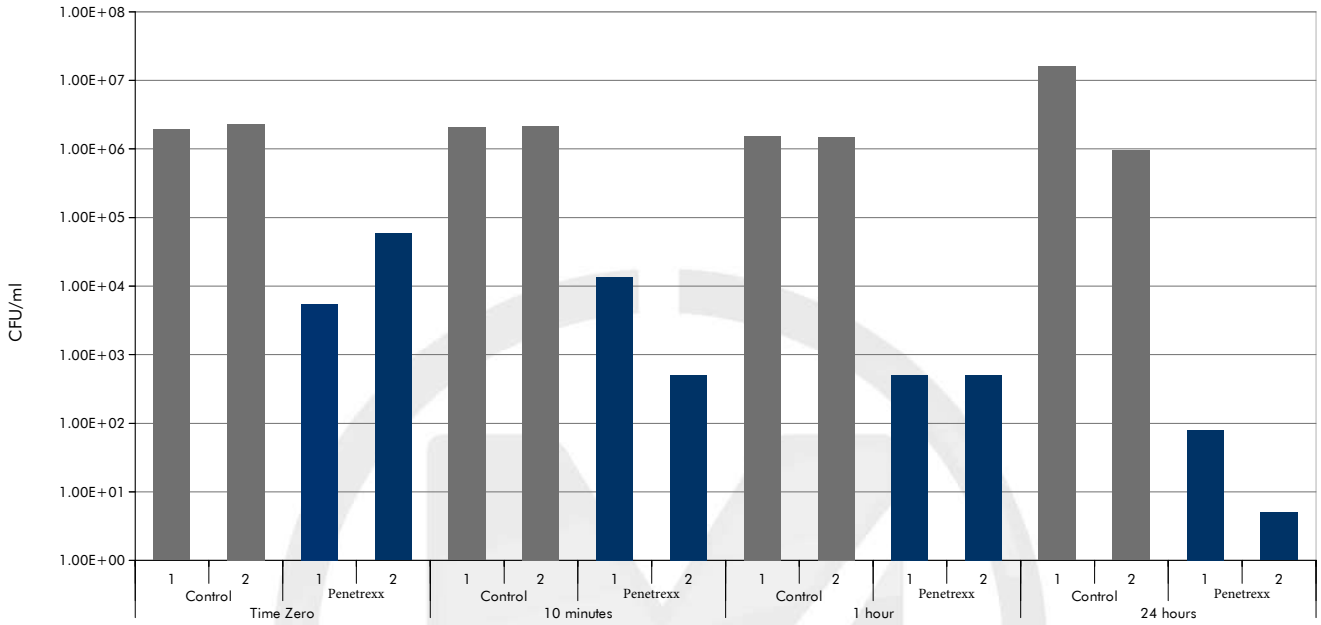
1304 W. INDUSTRIAL BLVD. ROUND ROCK, TX 78681 | © 2016 | CONTACT@MICROCHEMLAB.COM | 512.310.8378

Results of the Study

Test Microorganism	Contact Time	Test Substance	Replicate	CFU/Carrier	Average CFU/Carrier	Average Percent Reduction Compared to Control at Time Zero	Average Log_{10} Reduction Compared to Control at Time Zero
S. enterica ATCC 10708	Time Zero	Control	1	1.90E+06	2.10E+06	N/A	
			2	2.30E+06			
		Penetrexx	1	5.50E+03	3.18E+04	98.49%	1.82
			2	5.80E+04			
	10 minutes	Control	1	2.05E+06	2.10E+06	None	
			2	2.15E+06			
		Penetrexx	1	1.35E+04	7.00E+03	99.67%	2.48
			2	5.00E+02			
	1 hour	Control	1	1.55E+06	1.53E+06	27.38%	0.14
			2	1.50E+06			
		Penetrexx	1	<5.00E+02*	<5.00E+-2	>99.98%	>3.62
			2	<5.00E+02*			
	24 hours	Control	1	1.58E+07	8.35E+06	None	
			2	9.50E+05			
		Penetrexx	1	8.00E+01	4.25E+01	99.998%	4.69
			2	5.00E+00			

**The limit of detection for the "control" in this assay is 5.00E+02. Values below the limit of detection are reported at <5.00E+02 in the table.*

Results of the Study (cont.)



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