ANTIMICROBIAL TEST LABORATORIES

Study Report

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Study Title

Antibacterial Efficacy of Bio-Care Technology's Non-Porous Test Substance

Test Method

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

Study Identification Number

NG6470

Study Sponsor

Bio-Care Technology

Test Facility

Antimicrobial Test Laboratories
1304 W. Industrial Blvd
Round Rock, TX 78681
(512) 310-8378

History of the Laboratory

Antimicrobial Test Laboratories was launched in 2006 to provide testing services to the antimicrobial industry. The company has grown considerably since then but its focus remains the same: Test antimicrobial agents, test them well, and test them fast! Antimicrobial Test Laboratories operates a 15,000+ square foot facility near Austin, Texas, where hundreds of studies are conducted annually by a staff of friendly, knowledgeable, and experienced microbiologists and virologists.

Laboratory Qualification Statement

Antimicrobial Test Laboratories was founded by microbiologist Dr. Benjamin Tanner. The laboratory ensures consistent, reproducible results by utilizing a well-trained and educated scientific staff who work from a comprehensive system of Standard Operating Procedures, official standard methods from ASTM, AOAC, and other organizations, and custom study protocols. The laboratory provides testing services to dozens of Fortune 500 companies and has been inspected for GLP compliance by the US government.

Scientist Qualifications

Your Study was designed, conducted and reported by: Alex Vazquez, B.S.

Alex graduated from University of Texas at El Paso with a Bachelors of Science degree in Microbiology.

Alex is hardworking and highly dedicated to all projects he is assigned. His great customer service skills aid him to exceed sponsor expectations and produce exceptional work. As a Microbiology Associate at Antimicrobial Test Laboratories, his attention to detail and drive for perfection make him an asset to both the sponsor and the laboratory.

If you have any questions about your study, please don't hesitate to contact Alex at:

Alex@AntimicrobialTestLabs.com
or
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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

Antimicrobial Test Laboratories 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 Antimicrobial Test Laboratories 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
04NOV2015 *C. difficile* Spore Prep.	05NOV2015	05NOV2015	06NOV2015	09NOV2015	10NOV2015

Test Substance Information

The test substance was received on 04 NOV 2015 and the following picture was taken.
(note: photo depicts the side/face of test substance that was analyzed in this study)

Test Substance Received: Panel #1

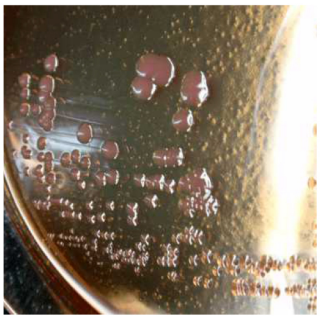
Test Substance arrived in dimension that was not optimal for the conduct of the study. Test substance was cut down to ideal size for the study.

Test Microorganism Information

The test microorganism(s) selected for this test:

***Pseudomonas aeruginosa* 9027**

This bacteria is a Gram-negative, rod-shaped microorganism with a single flagellum. It grows optimally under aerobic conditions, however, it can use a host of electron receptors to respire anaerobically. *P. aeruginosa* can be found almost anywhere in nature and it is an opportunistic pathogen. Like many other bacterial-related diseases, the ability to form resilient biofilms within human tissues under anaerobic conditions is thought to be the primary cause for pathogenicity.

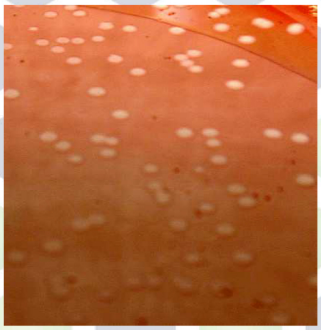


***Acinetobacter baumannii* 19606**

This bacteria is a Gram-negative, rod-shaped aerobe. *A. baumannii* can be responsible for infections such as pneumonia and septicemia in immunodeficient patients. Multi-drug resistant *A. baumannii* is a growing concern, especially in hospital settings, where it is thought that the bacterium can survive on hospital surfaces for long periods. *A. baumannii*'s ability to survive and avoid desiccation contribute to this microbe's fitness and can make this bacterium relatively difficult to disinfect.

***Enterobacter cloacae* (CRE) BAA-2468**

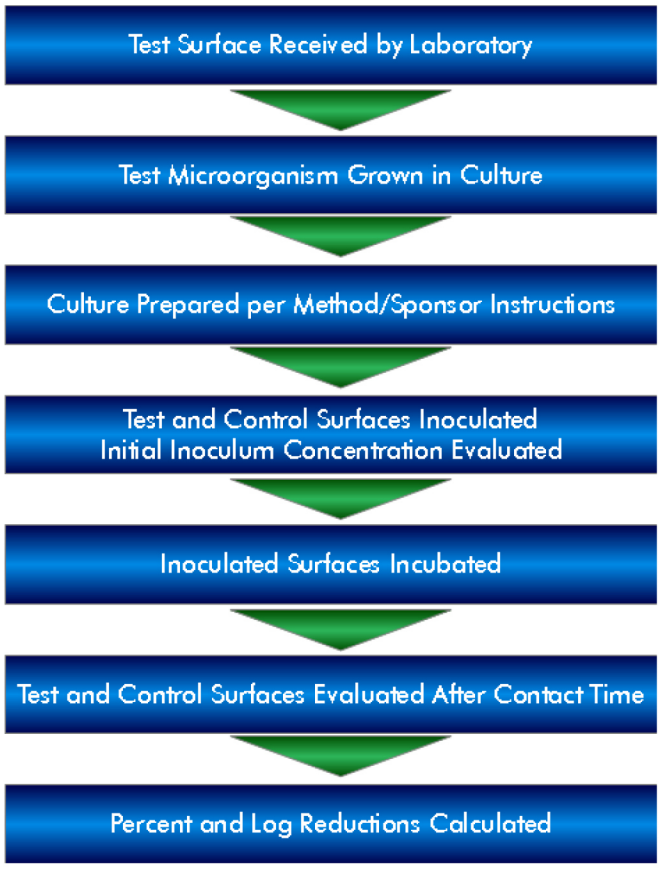
This bacteria is a Gram-negative, rod shaped, facultative anaerobe commonly found in the gastrointestinal tract of humans. It is not usually a primary pathogen although it is sometimes associated with urinary and respiratory tract infections. Although relatively susceptible to disinfection and desiccation when dried on a surface, it can be a challenging microorganism to mitigate in solution.



***Clostridium difficile* 43598 (endospores)**

This bacteria is a Gram-positive, rod shaped, endospore generating obligate anaerobe. *Clostridium* species are part of the normal human gut flora that produce spores which are highly resistant to chemical and environmental conditions. *C. diff* is commonly associated with hospital acquired infections and is know to cause antibiotic assisted colitis. Because of it's high resistance to antimicrobials, *C. difficile* is a benchmark bacteria for sporicidal and sterilant activity of chemicals.

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 Antimicrobial Test Laboratories 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):	40 mm x 40 mm
Replicates:	One		

Culture Growth Media:	TSB, Spore Prep.	Culture Growth Time:	18 hours
Culture Dilution Media:	1:500 NTB	Culture Dilution Supplement:	N/A
Inoculum Concentration:	~6.0 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:	TSA, BHIY-HT
Enumeration Plate Incubation Temperature:	36°C ± 1°C	Enumeration Plate Incubation Time:	24-48 Hours

Study Modifications

No modifications were made for this study.

Study Notes

No notes were made for this study.

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Control Results

Neutralization Method: N/A Media Sterility: Sterile
Growth Confirmation: Confirmed via Morphology on TSA and BHIY-HT

Calculations

$$\text{Percent Reduction} = \left(\frac{B - A}{B}\right) \times 100$$

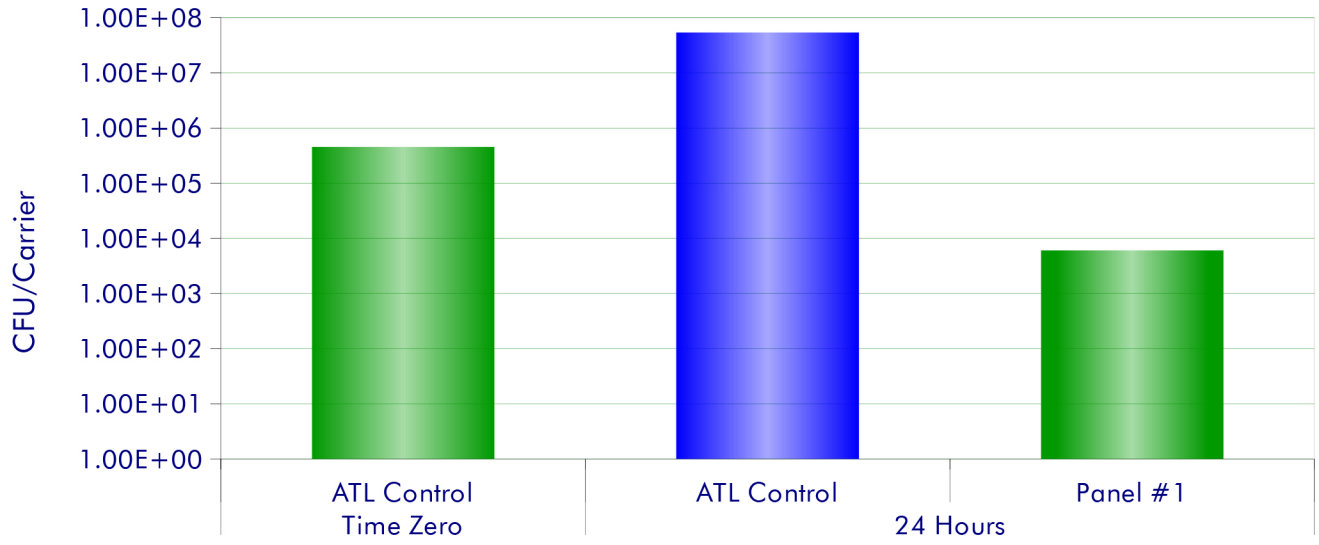
Where:
B = Number of viable test microorganisms on the control carriers after the contact time
A = Number of viable test microorganisms on the test carriers after the contact time

$$\text{Log}_{10}\text{Reduction} = \text{Log}\left(\frac{B}{A}\right)$$

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

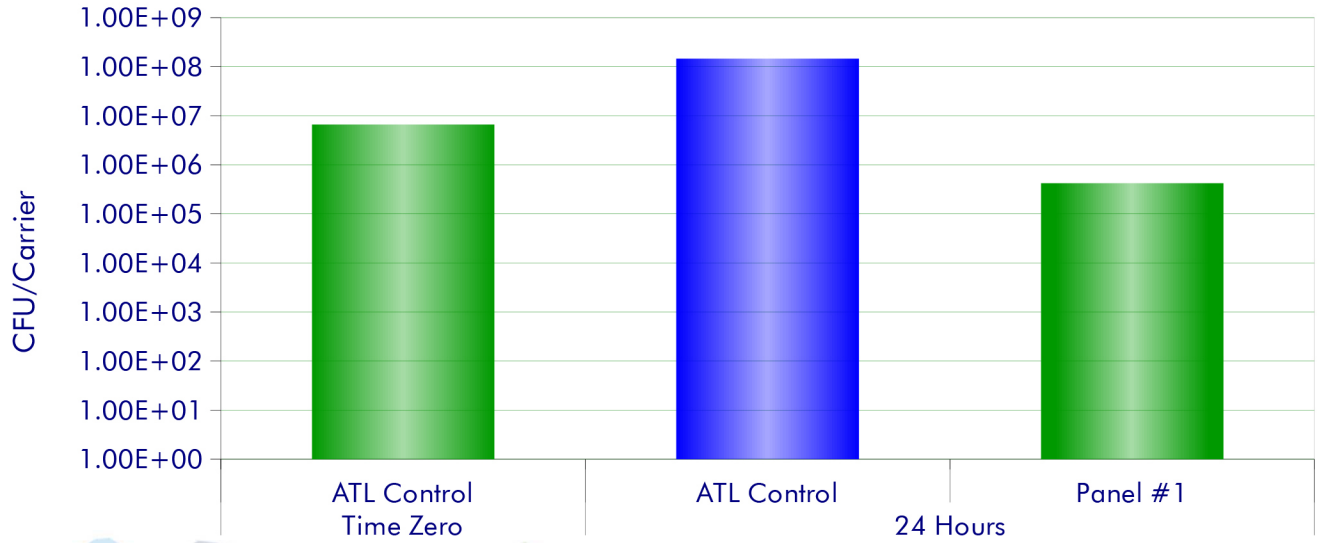
Results of the Study for *P. aeruginosa* 9027

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
P.aeruginosa 9027	Time Zero	ATL Control	4.50E+05	N/A	
	24 Hours	ATL Control	5.34E+07		
		Panel #1	6.00E+03	99.99%	3.95



Results of the Study for *A. baumannii* 19606

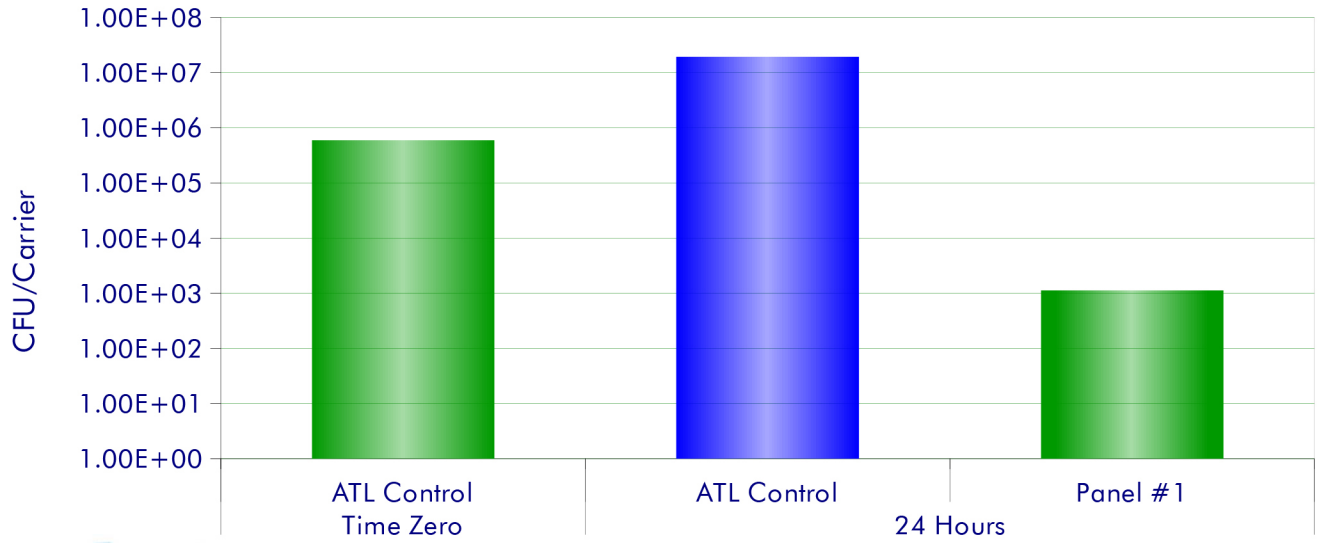
Test Microorganism	Contact Time	Carrier Type	CFU/Carrier	Percent Reduction Compared to Control at Contact Time	Log_{10} Reduction Compared to Control at Contact Time
A. baumannii 19606	Time Zero	ATL Control	6.55E+06	N/A	
	24 Hours	ATL Control	1.46E+08		
		Panel #1	4.18E+05	99.71%	2.54



Results

Results of the Study for *E. cloace* (CRE) BAA-2468

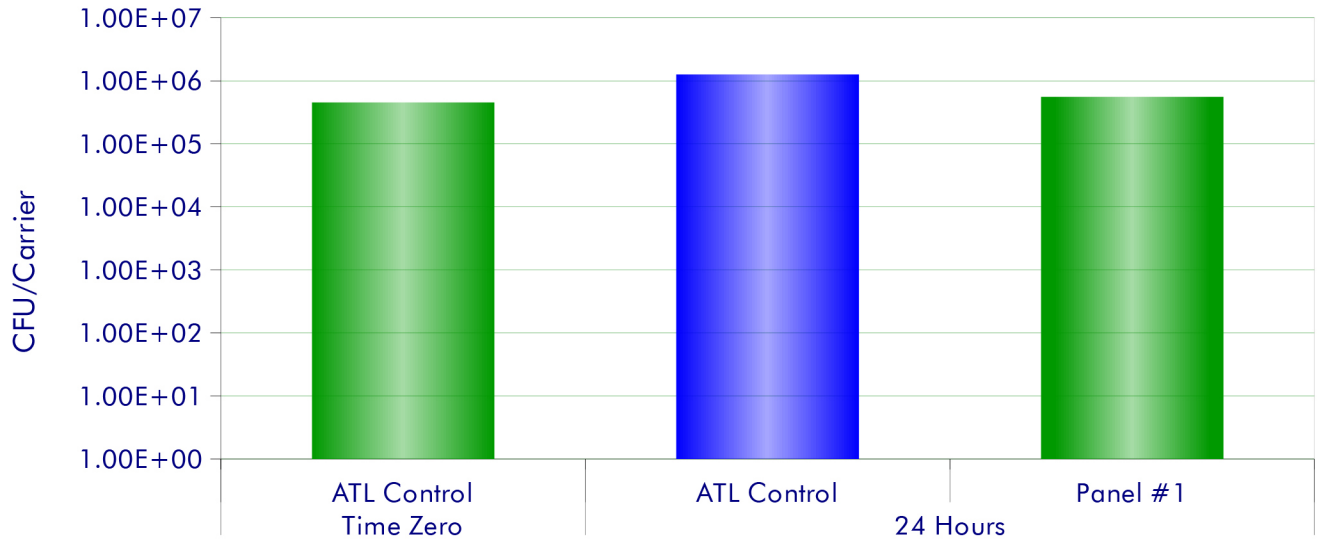
Test Microorganism	Contact Time	Carrier Type	CFU/Carrier	Percent Reduction Compared to Control at Contact Time	Log_{10} Reduction Compared to Control at Contact Time
E. cloace (CRE) BAA-2468	Time Zero	ATL Control	5.90E+05	N/A	
	24 Hours	ATL Control	1.93E+07		
		Panel #1	1.12E+03	99.99%	4.24



Results

Results of the Study for *C. difficile* 43598

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
C. difficile 43598	Time Zero	ATL Control	4.50E+05	N/A	
	24 Hours	ATL Control	1.25E+06		
		Panel #1	5.50E+05	56.00%	0.36



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