ANTIMICROBIAL TEST LABORATORIES

Study Report

www.AntimicrobialTestLabs.com - Info@AntimicrobialTestLabs.com - (512) 310-8378

Study Title

Antibacterial Activity and Efficacy of Chemiplastica SA Non-porous Test Substance

Test Method

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

Study Identification Number

NG5280

Study Sponsor

Emanuele Verga
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Test Facility

Antimicrobial Test Laboratories
1304 W. Industrial Blvd
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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: Blake Roland, BS and Diego Ugarte, BS

Blake graduated from the University of Oklahoma with a Bachelors of Science in Microbiology.

Blake is well-versed with regard to a variety of microbiological test methods and procedures. As a Microbiologist at Antimicrobial Test Laboratories, he has taken part in hundreds of studies and mastered several test methods. Blake enjoys seeing large projects through to completion. His scientific character, coupled with his strong work ethic bring a high degree of efficiency and care to every study he leads.

Diego graduated from the University of Texas with a Bachelors of Science in Microbiology.

Diego is an experienced microbiologist and energetic professional. He has mastered many test methods, including complex methods such as EPA's residual self sanitization procedure. As a Microbiologist at Antimicrobial Test Labs, Diego conducts studies efficiently and consistently. He understands antimicrobial product regulations and is known in the laboratory for his work ethic, interest in projects, and general diligence.

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

Blake@AntimicrobialTestLabs.com
or
Diego@AntimicrobialTestLabs.com
or
(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

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

E. coli 8739 and *C. albicans* 10231	15JUL2014	16JUL2014	16JUL2014	17JUL2014	18JUL2014
S. aureus 33592 (MRSA) and *E. faecalis* 19433	22JUL2014	23JUL2014	23JUL2014	24JUL2014	25JUL2014
A. baumannii 19606, *P. aeruginosa* 9027 and *E. aerogenes* 13048	06AUG2014	07AUG2014	07AUG2014	08AUG2014	09AUG2014
S. aureus 6538 and *S. marcescens* 13880	10AUG2014	11AUG2014	11AUG2014	12AUG2014	13AUG2014

14AUG2014

Test Substance Information

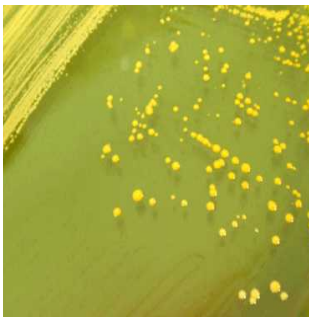
The test substance was received on 27 JUN 2014 and the following pictures were taken.

Test Substances Received: Urochem Moulding Compound

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

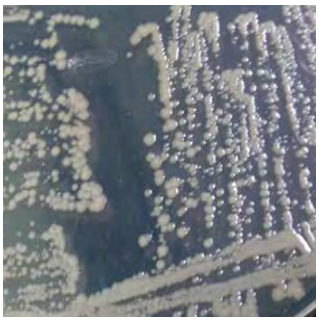
Test Microorganism Information

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.

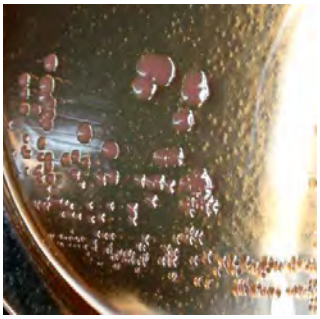
Candida albicans 10231

This fungi is facultatively aerobic and can grow both as a yeast and as a filamentous fungus. *Candida albicans* is a commensal microorganism meaning it normally inhabits the human mouth and gastrointestinal tract but is opportunistic and can cause candidiasis or thursh. *Candida albicans* can survive for long periods of time without nutrients and is known to form biofilms on medical devices, therefore, disinfection to kill these fungi is very important.

Enterococcus faecalis 19433

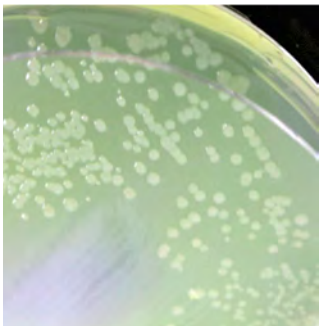
This bacteria is a Gram-positive, spherical-shaped strain of *Enterococcus faecalis* that has developed resistance to the antibiotic vancomycin. *E. faecalis* (VRE) can cause a variety of local and systemic infections including endocarditis, bacteremia, and urinary tract infections, which are exceptionally difficult to treat because of this strain's acquired drug-resistance. Due to this bacterium's robust survival factors and resistance to commonly used antimicrobial agents, this bacterium is very challenging to disinfect.

Test Microorganism Information



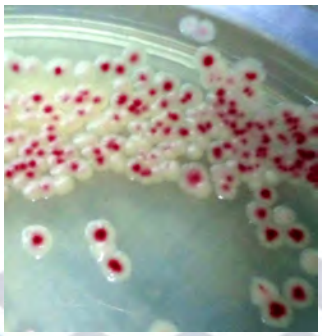
Acinetobacter baumannii 19606

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.



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.



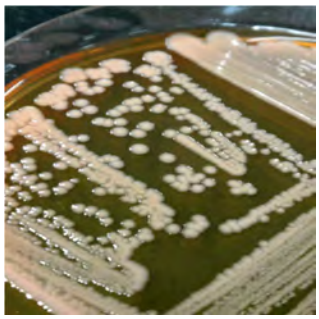
Serratia marcescens 13880

This bacteria is a Gram-negative, rod-shaped, facultative anaerobe that has been classified as an opportunistic pathogen. *S. marcescens* can be responsible for infections at several sites on the human body including the eyes, urinary tract, and respiratory system. This bacteria can be commonly found in damp environments like bathrooms, where it manifests as a pink-orange film due to a reddish-orange pigment called prodigiosin.

Staphylococcus aureus (MRSA) 33592

This bacteria is a Gram-positive, cocci shaped, aerobe which is resistant to the penicillin-derivative antibiotic methicillin. MRSA can cause troublesome infections, and their rapid reproduction and resistance to antibiotics makes them more difficult to treat. MRSA bacteria are resistant to drying and can therefore survive on surfaces and fabrics for an extended period of time and therefore makes this bacteria an excellent representative for antimicrobial efficacy testing on surfaces.

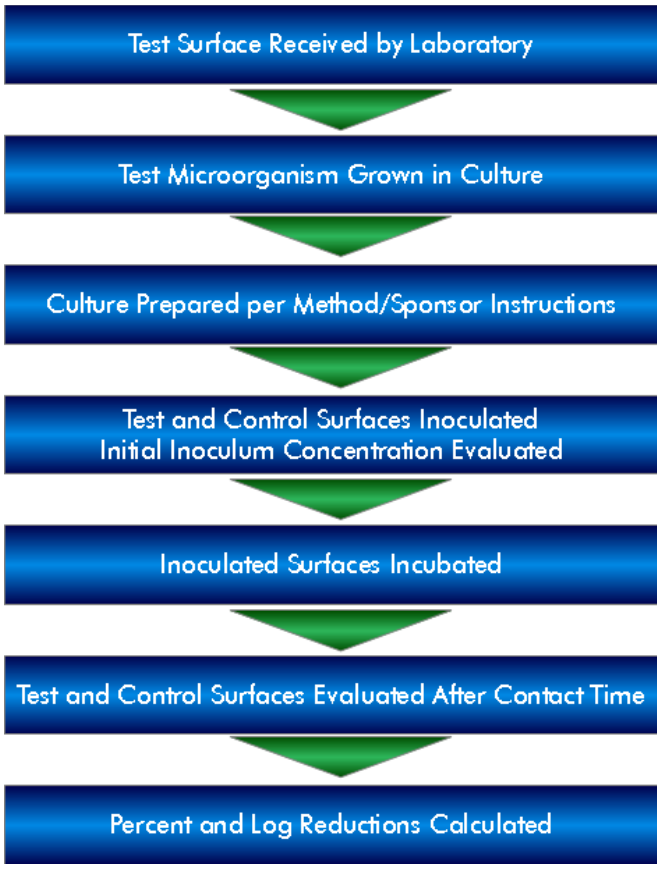
Test Microorganism Information



***Enterobacter aerogenes* 13048**

This bacteria is Gram-negative, rod-shaped, and radially surrounded by flagellum. It can be found in dairy products, soil, and the gastrointestinal tract of animals. *E. aerogenes* is related to many bacteria including, *Escherichia*, *Klebsiella*, *Shingella*, and *Serratia*. This bacteria can be involved in urinary tract, gastrointestinal, and bloodstream infections, and is implicated as a potential cause adult meningitis. Because of it's common association with human illness, *E. aerogenes* is frequently used as a benchmark for disinfectant efficacy.

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:	40 mm x 40 mm	Film Used? (Size):	Yes (40 mm x 40 mm)
Replicates:	Three		

Culture Growth Media:	TSB and PDA	Culture Growth Time:	18 hours
Culture Dilution Media:	1:500 Nutrient Broth	Culture Dilution Supplement:	N/A
Inoculum Concentration:	2.3 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 and PDA
Enumeration Plate Incubation Temperature:	36°C ± 1°C	Enumeration Plate Incubation Time:	24-48 hours

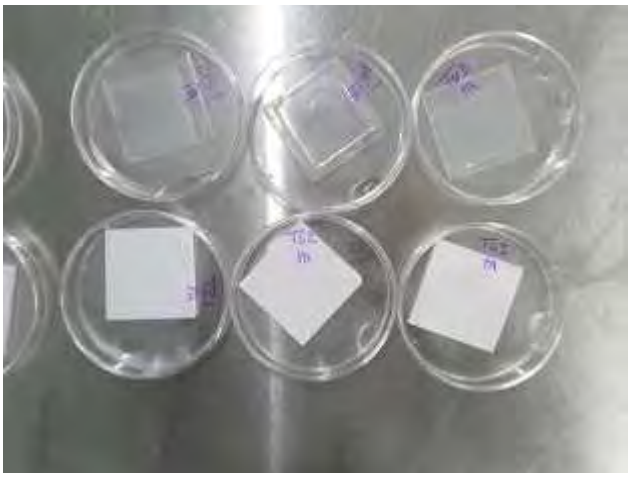
Study Modifications

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

Study Notes

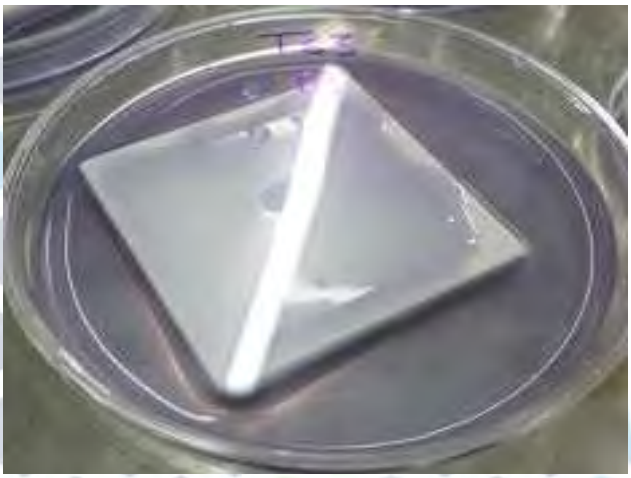
No additional observations or notations were made for this study.

Study Photographs

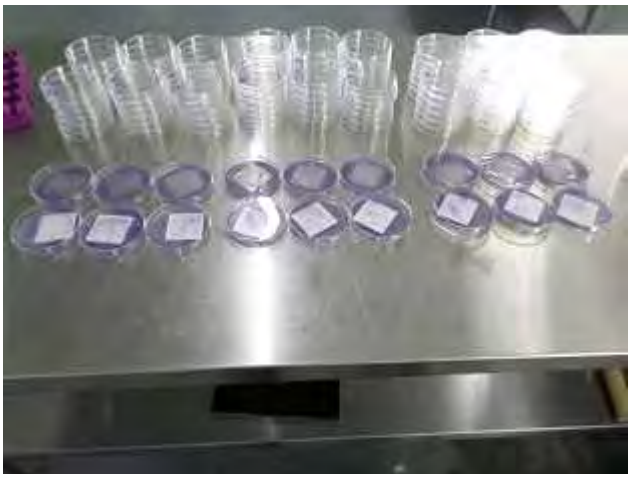


Inoculated Control (Top) and Test (bottom) Samples prior to incubation

Control and Test Samples after 24 hour incubation



Neutralized Test Sample after 24 hour contact time



Neutralized Control and Test Samples after 24 hour incubation

Control Results

Neutralization Method: Validated
Growth Confirmation: Confirmed
Media Sterility: Sterile

Calculations

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

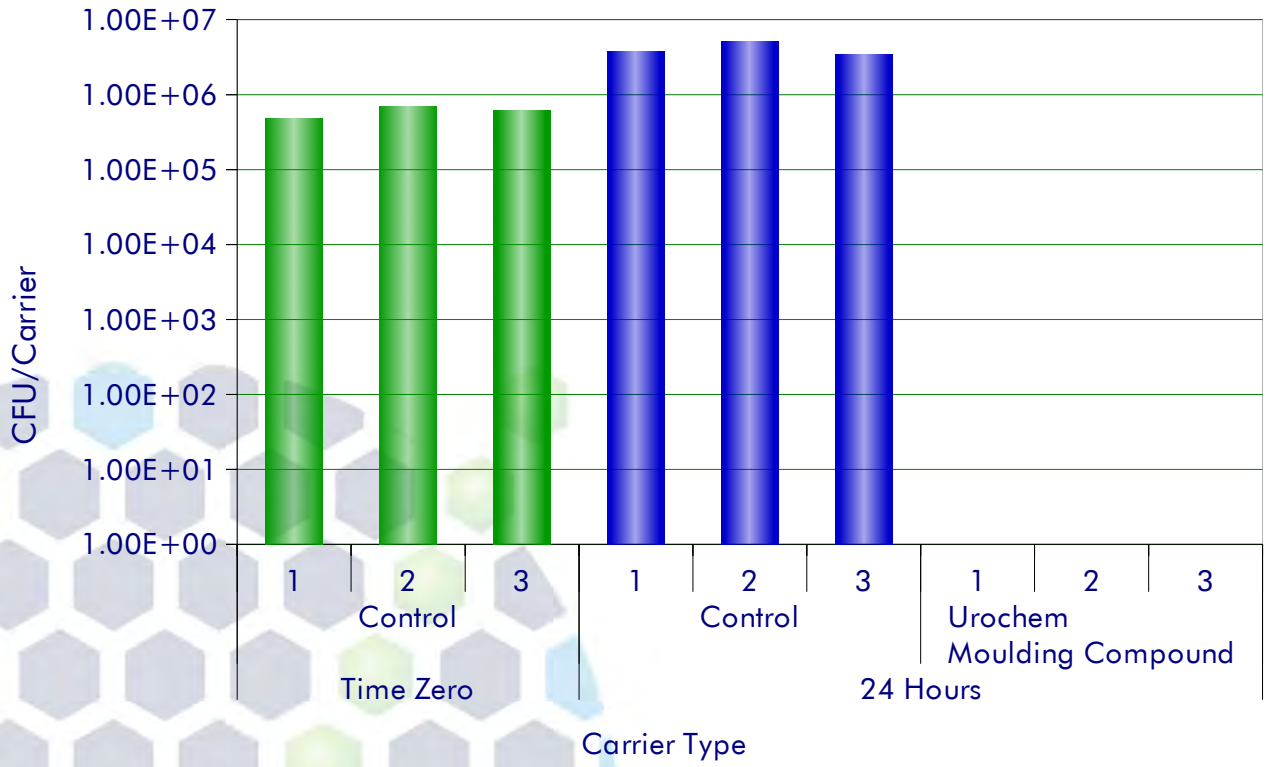
Where:
B = Number of viable test microorganisms on the control carriers after the contact time
A = Number of viable test microorganisms on the test carriers after the contact time

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

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

Results of the Study

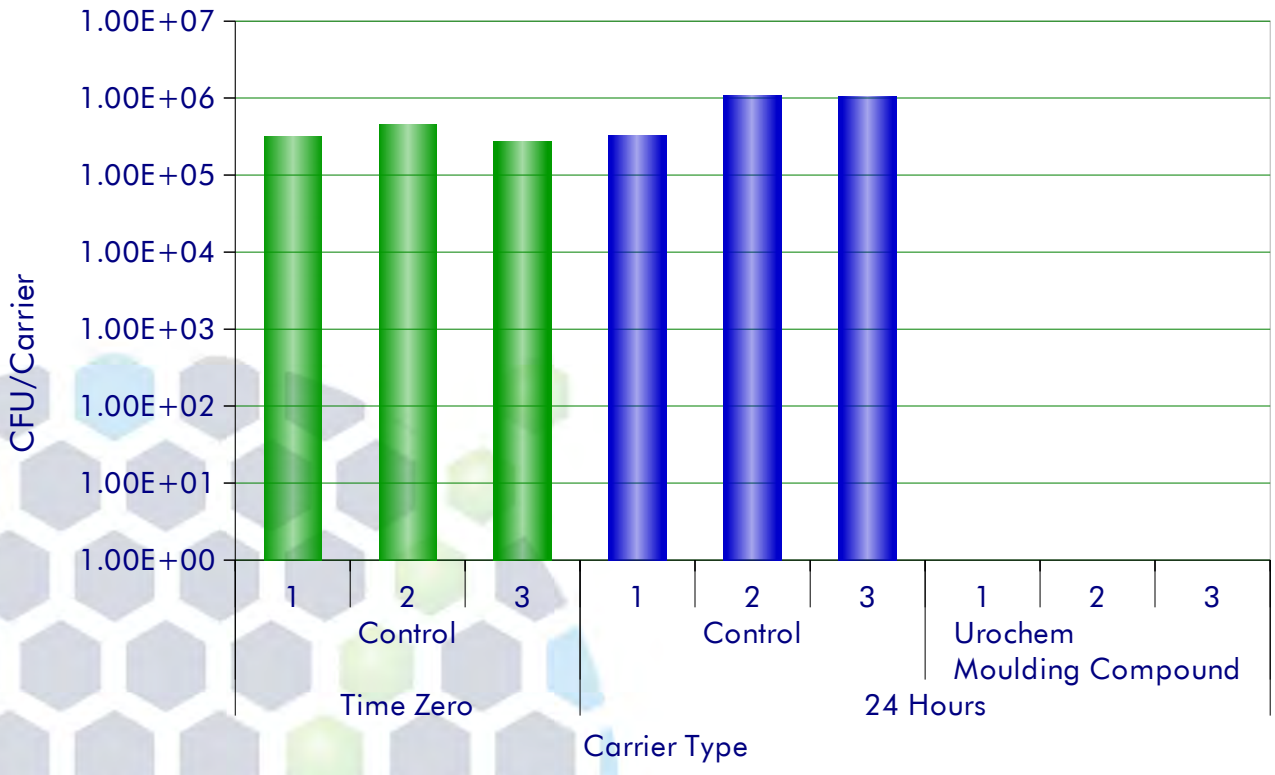
Test Microorganism	Contact Time	Carrier Type	CFU/Carrier	Average CFU/Carrier	Percent Reduction Compared to Control at Contact Time	Log_{10} Reduction Compared to Control at Contact Time
S. aureus 6538	Time Zero	Control	4.75E+05	5.87E+05	N/A	
			6.80E+05			
			6.05E+05			
	24 Hours	Control	3.70E+06	2.82E+06		
			5.05E+06			
			3.40E+06			
		Urochem Moulding Compound	<5.00E+00	<5.00E+00	>99.9998%	>5.75
			<5.00E+00			
			<5.00E+00			



The limit of detection for this study is 5 CFU/ml. Values less than the limit of detection are presented as <5.00E+00 in the table and zero on the graph above.

Results of the Study

Test Microorganism	Contact Time	Carrier Type	CFU/Carrier	Average CFU/Carrier	Percent Reduction Compared to Control at Contact Time	Log_{10} Reduction Compared to Control at Contact Time
E. coli 8739	Time Zero	Control	3.20E+05	3.50E+05	N/A	
			4.55E+05			
			2.75E+05			
	24 Hours	Control	3.30E+05	8.27E+05		
			1.10E+06			
			1.06E+06			
		Urochem Moulding Compound	<5.00E+00	<5.00E+00	>99.9994%	>5.22
			<5.00E+00			
			<5.00E+00			



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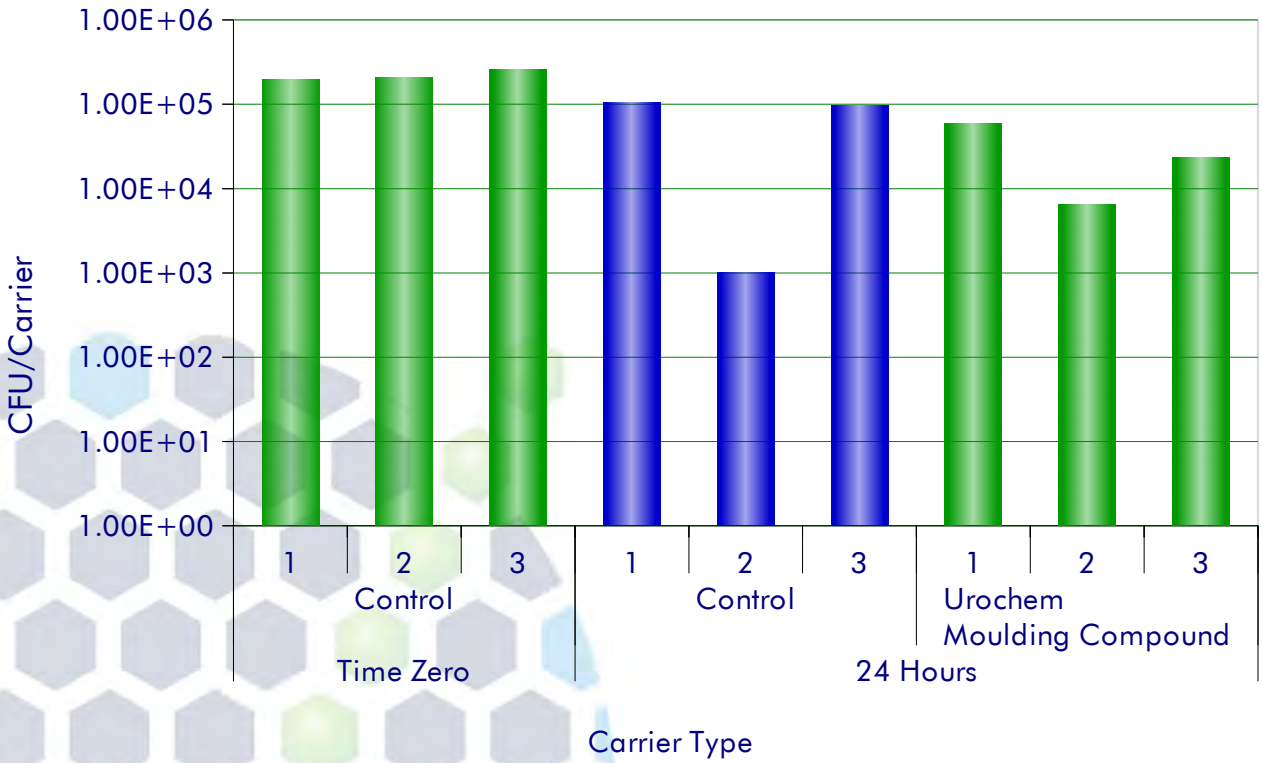
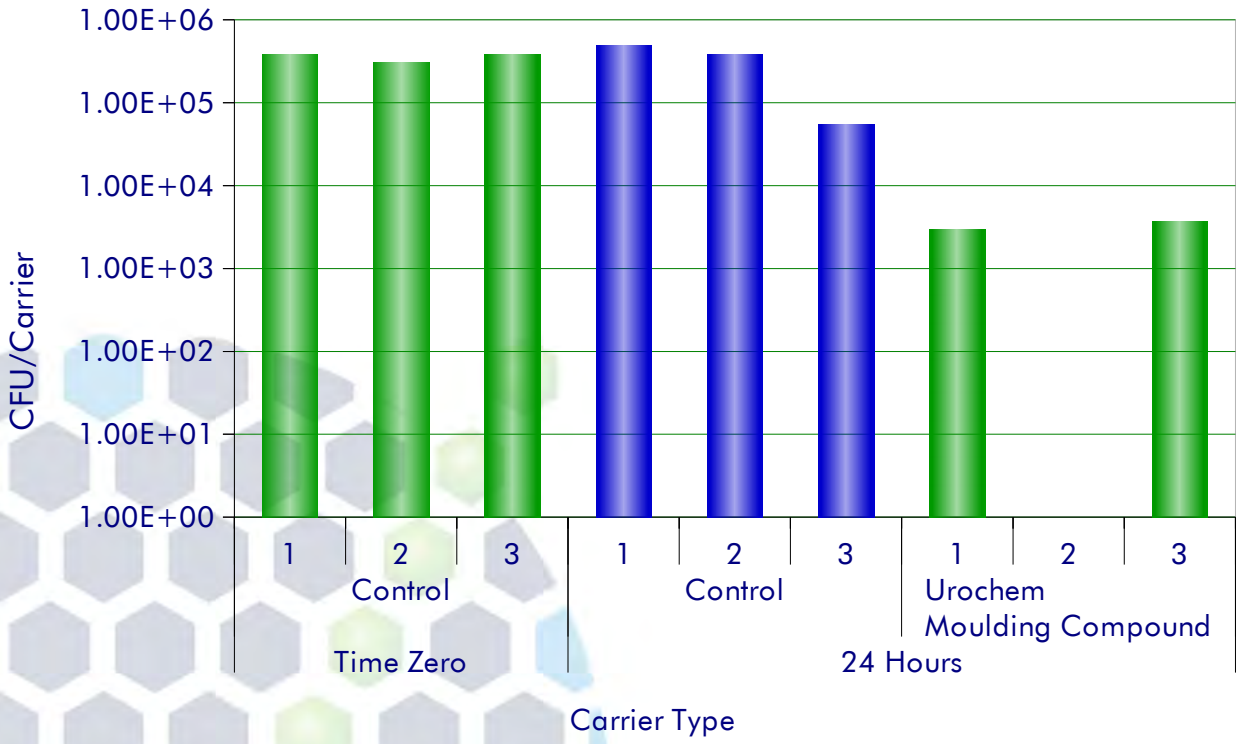
Results of the Study

Test Microorganism	Contact Time	Carrier Type	CFU/Carrier	Average CFU/Carrier	Percent Reduction Compared to Control at Contact Time	Log_{10} Reduction Compared to Control at Contact Time
C. albicans 10231	Time Zero	Control	1.95E+05	2.18E+05	N/A	
			2.05E+05			
			2.55E+05			
	24 Hours	Control	1.05E+05	6.68E+04		
			1.00E+03			
			9.50E+04			
		Urochem Moulding Compound	5.80E+04	2.92E+04	56.36%	0.36
			6.50E+03			
			2.30E+04			



Results of the Study

Test Microorganism	Contact Time	Carrier Type	CFU/Carrier	Average CFU/Carrier	Percent Reduction Compared to Control at Contact Time	Log_{10} Reduction Compared to Control at Contact Time
E. faecalis 19433	Time Zero	Control	3.75E+05	3.52E+05	N/A	
			3.05E+05			
			3.75E+05			
	24 Hours	Control	4.90E+05	3.07E+05		
			3.75E+05			
			5.45E+04			
		Urochem Moulding Compound	2.92E+03	<2.21E+03	>99.28%	>2.14
			<5.00E+00			
			3.70E+03			



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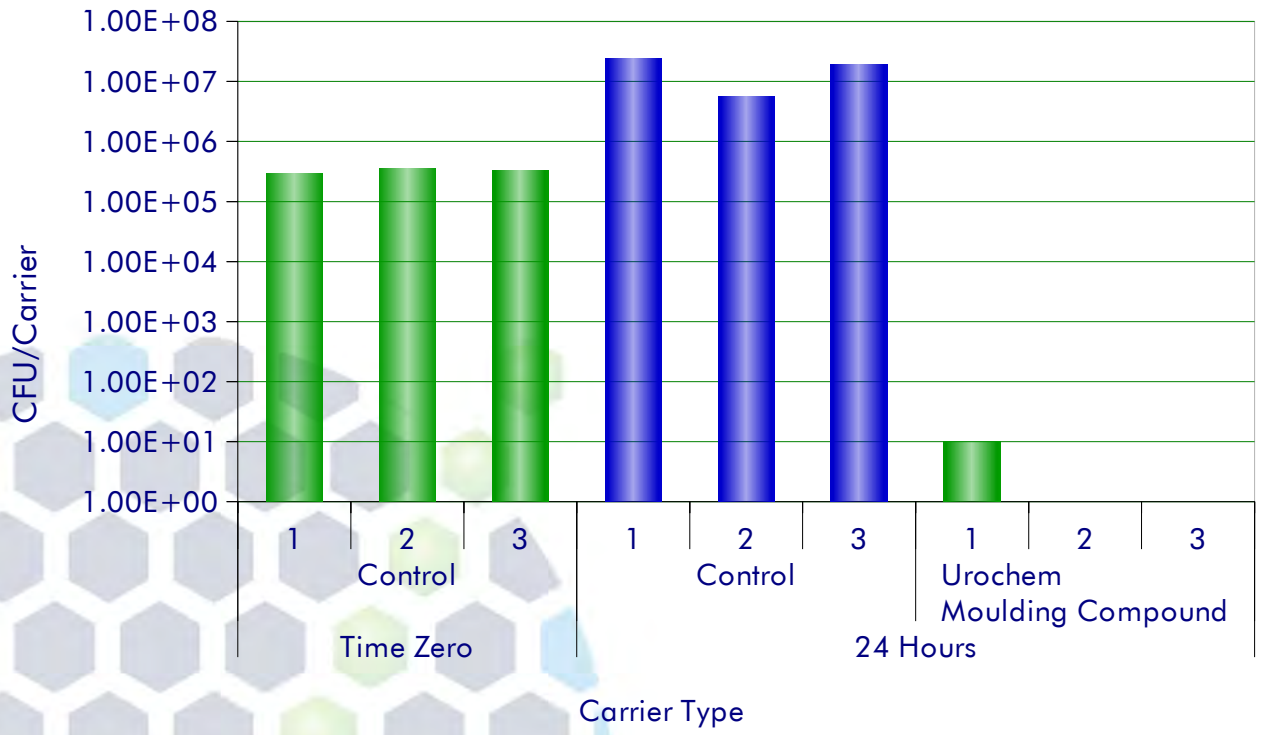
Results of the Study

Test Microorganism	Contact Time	Carrier Type	CFU/Carrier	Average CFU/Carrier	Percent Reduction Compared to Control at Contact Time	Log_{10} Reduction Compared to Control at Contact Time
A. baumannii 19606	Time Zero	Control	2.85E+05	3.23E+05	N/A	
			3.55E+05			
			3.30E+05			
	24 Hours	Control	2.41E+07	1.62E+07		
			5.50E+06			
			1.90E+07			
		Urochem Moulding Compound	1.00E+01	<6.67E+00	>99.99996%	>3.89
			<5.00E+00			
			<5.00E+00			



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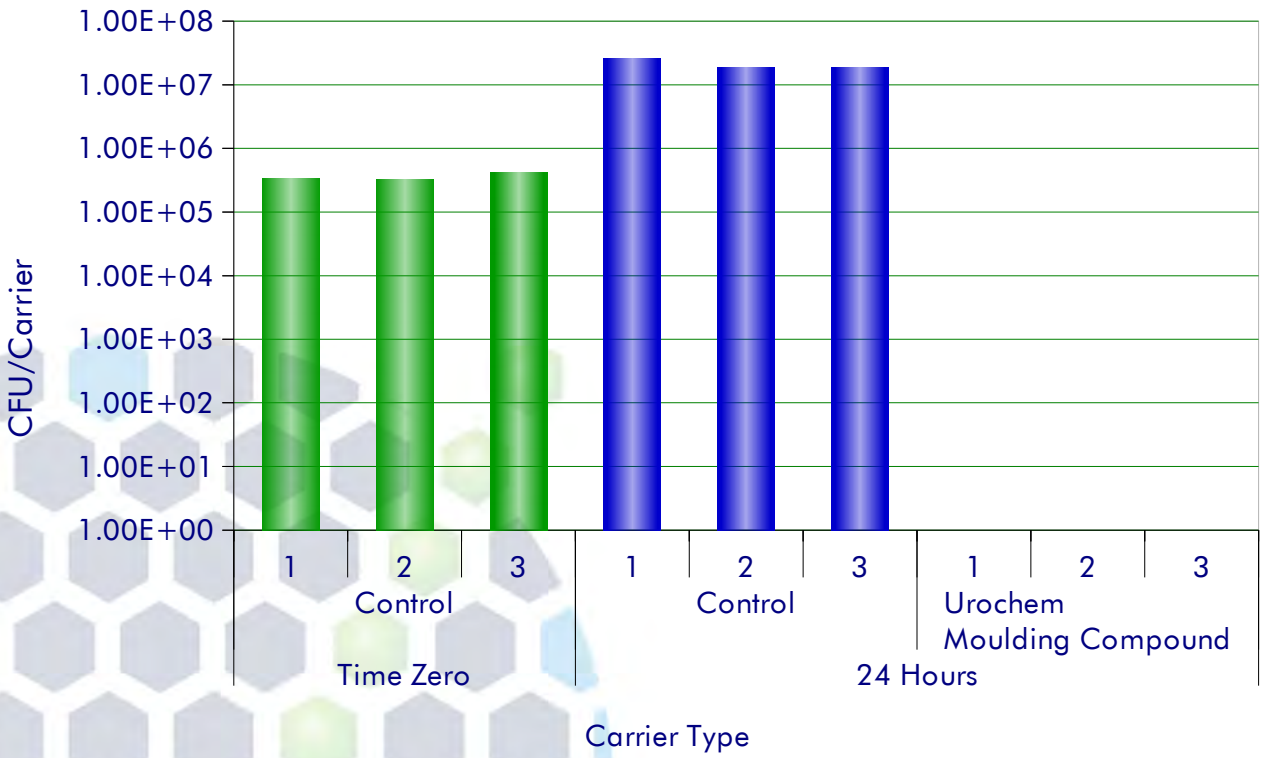
Results of the Study

Test Microorganism	Contact Time	Carrier Type	CFU/Carrier	Average CFU/Carrier	Percent Reduction Compared to Control at Contact Time	Log_{10} Reduction Compared to Control at Contact Time
P. aeruginosa 9027	Time Zero	Control	3.35E+05	3.57E+05	N/A	
			3.25E+05			
			4.10E+05			
	24 Hours	Control	2.56E+07	2.08E+07		
			1.84E+07			
			1.84E+07			
		Urochem Moulding Compound	<5.00E+00	<5.00E+00	>99.99998%	>6.62
			<5.00E+00			
			<5.00E+00			



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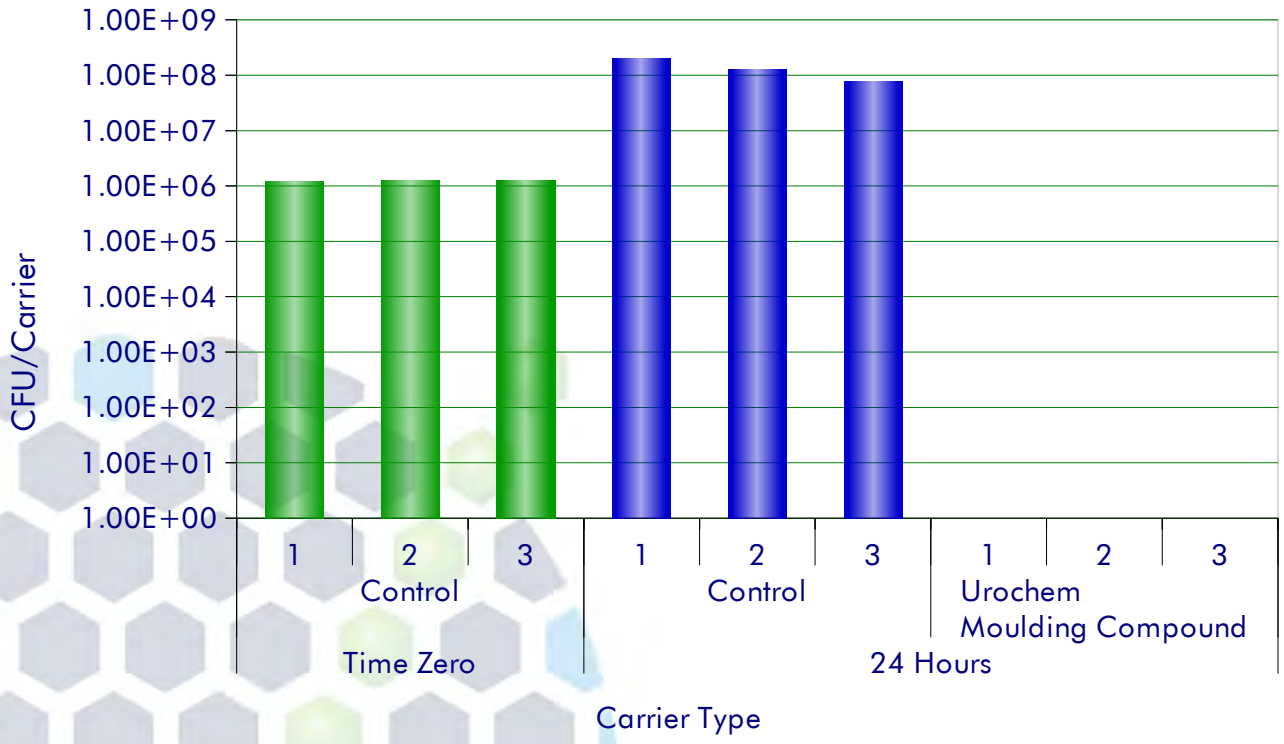
Results of the Study

Test Microorganism	Contact Time	Carrier Type	CFU/Carrier	Average CFU/Carrier	Percent Reduction Compared to Control at Contact Time	Log_{10} Reduction Compared to Control at Contact Time
S. marcescens 13880	Time Zero	Control	1.20E+06	1.23E+06	N/A	
			1.25E+06			
			1.25E+06			
	24 Hours	Control	1.99E+08	1.34E+08		
			1.26E+08			
			7.60E+07			
		Urochem Moulding Compound	<5.00E+00	<5.00E+00	>99.999996%	>7.43
			<5.00E+00			
			<5.00E+00			



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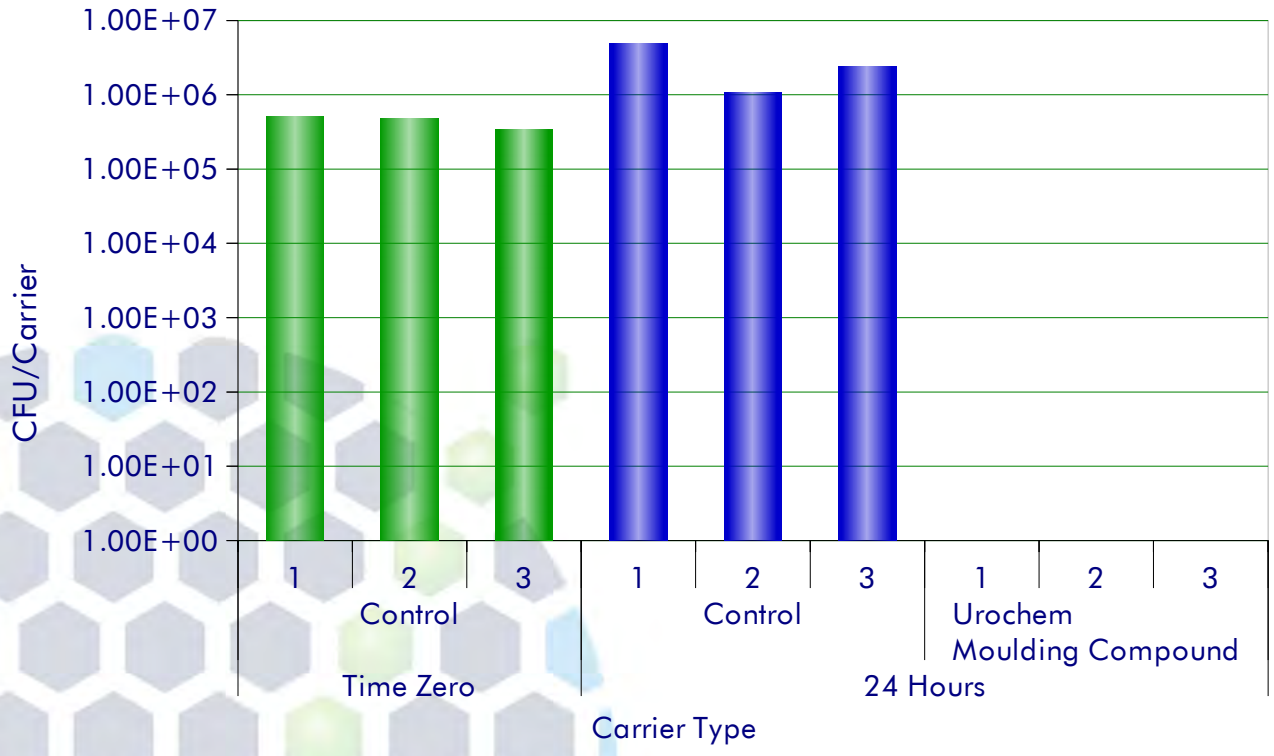
Results of the Study

Test Microorganism	Contact Time	Carrier Type	CFU/Carrier	Average CFU/Carrier	Percent Reduction Compared to Control at Contact Time	Log_{10} Reduction Compared to Control at Contact Time
S. aureus 33592	Time Zero	Control	5.10E+05	4.42E+05	N/A	
			4.75E+05			
			3.40E+05			
	24 Hours	Control	4.85E+06	2.77E+06		
			1.05E+06			
			2.40E+06			
		Urochem Moulding Compound	<5.00E+00	<5.00E+00	>99.99982%	>5.74
			<5.00E+00			
			<5.00E+00			



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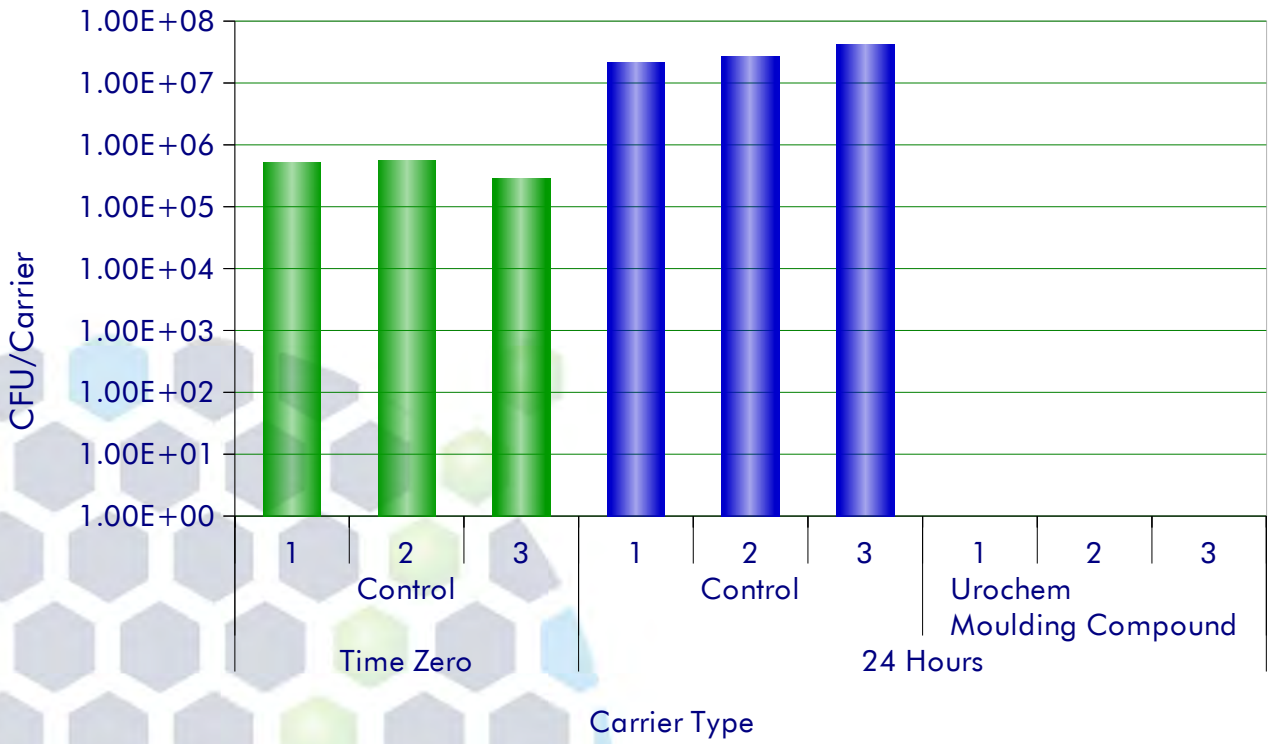
Results of the Study

Test Microorganism	Contact Time	Carrier Type	CFU/Carrier	Average CFU/Carrier	Percent Reduction Compared to Control at Contact Time	Log_{10} Reduction Compared to Control at Contact Time
E. faecalis 13048	Time Zero	Control	5.10E+05	4.52E+05	N/A	
			5.60E+05			
			2.85E+05			
	24 Hours	Control	2.12E+07	2.98E+07		
			2.66E+07			
			4.16E+07			
		Urochem Moulding Compound	<5.00E+00	<5.00E+00	>99.99998%	>6.78
			<5.00E+00			
			<5.00E+00			



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