MICROCHEM LABORATORY

STUDY REPORT

Study Title

Antibacterial Activity and Efficacy of Global Health RX's Test Substance
Using a Suspension Time-Kill Procedure

Test Method

ASTM International Method E2315
Assessment of Antimicrobial Activity using a Time-Kill Procedure

Study Identification Number

NG9518

Study Sponsor

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Test Facility

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ASTM E2315: General Information

ASTM International, formerly the American Society for Testing and Materials (ASTM), is an internationally recognized organization that develops and publishes product and testing standards. ASTM E2315 is a quantitative test method designed to assess changes in the population of microorganisms in an antimicrobial liquid suspension. The method is versatile and can be conducted using contact times ranging from ten seconds to 24 hours. The ASTM E2315 test method uses non-antimicrobial agents as controls to establish baselines for microbial reductions. Because ASTM E2315 allows a great degree of latitude with regard to how the procedure is carried out, some scientists consider it to be more similar to a testing guideline than a test method.

Study Timeline

Test Culture Initiated	Substances Inoculated	Substances Harvested	Substances Evaluated	Report Delivered
C. albicans				
24SEP2017	26SEP2017	26SEP2017 (6 hrs.) 27SEP2017 (24 hrs.)	28SEP2017	03OCT2017
A. niger				
07AUG2017	26SEP2017	26SEP2017 (6 hrs.) 27SEP2017 (24 hrs.)	28SEP2017	03OCT2017
E. coli, S. aureus, P. aeruginosa, S. aureus (MRSA), E. faecalis (VRE)				
25SEP2017	26SEP2017	26SEP2017 (6 hrs.) 27SEP2017 (24 hrs.)	28SEP2017	03OCT2017

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Test Substance Information

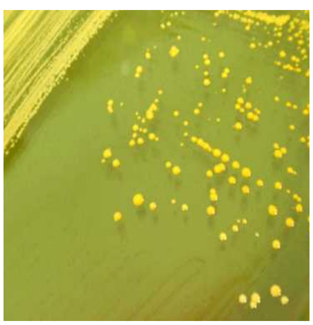
The test substances were received on 12SEP2017.
(note: photo depicts the test substance that was analyzed in this study)

Test substance: Hexagen wound dressing

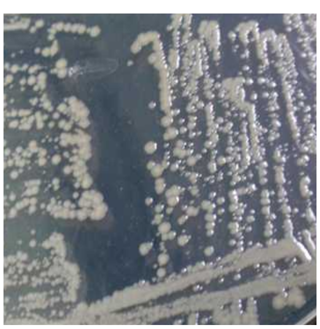
Test Substance arrived not ready to use for the conduct of the Study. Test substance was diluted for the study.

Test Microorganism Information

The test microorganism(s) selected for this test:



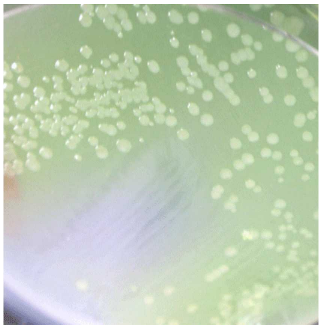
Staphylococcus aureus **6538**
This bacterium is a Gram-positive, spherical-shaped, facultative anaerobe. *Staphylococcus* species are known to demonstrate resistance to antibiotics such as methicillin. *S. aureus* pathogenicity can range from commensal skin colonization to more severe diseases such 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.

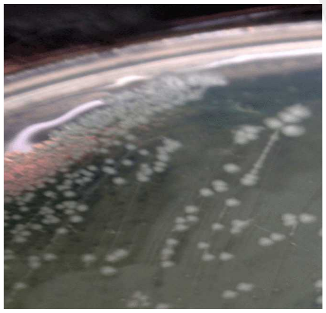
Test Microorganism Information (cont.)

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.

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 **(VRE) 51299**
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.

Aspergillus niger **9642**
This fungi is a conidiophore, or a sexual spore generating aerobic fungus. *A. niger* is responsible for food spoilage and black mold. Although it is primarily a plant pathogen, *A. niger* is known to cause the lung disease aspergillosis when a significant amount of spores are inhaled. *A. niger* is a fungus commonly associated with unsightly growth on manufactured goods and is commonly used as a benchmark for fungal resistance testing.

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Staphylococcus aureus (MRSA) 33591

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.

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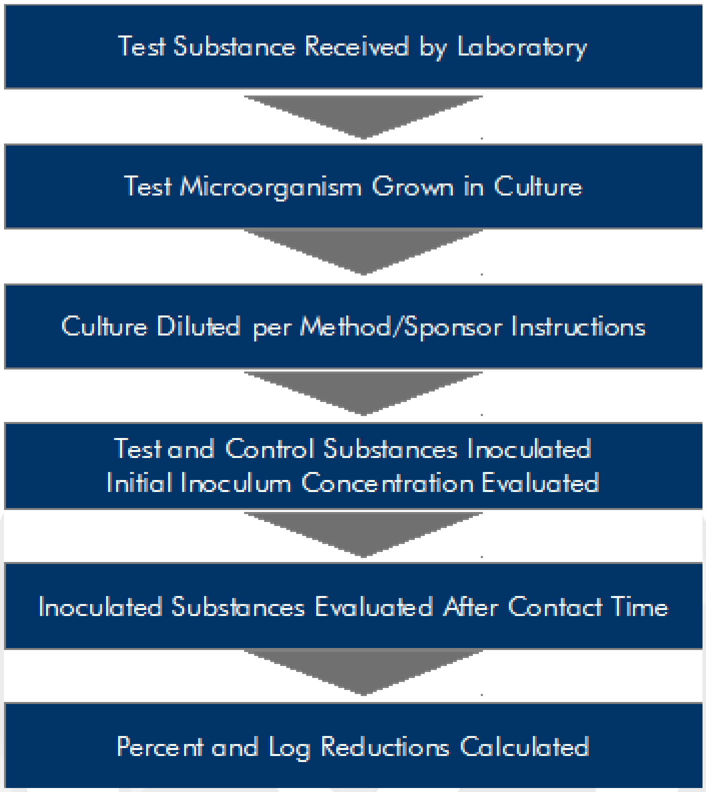
Diagram of the Procedure



Summary of the Procedure

- Test microorganisms are prepared in liquid culture medium for bacteria or on agar for fungi.
- The suspension of test microorganism is standardized, as needed, by dilution in a buffered saline solution.
- Test and control substances are dispensed in identical volumes to sterile vessels.
- Independently, Test and Control substances are inoculated with each test microorganism, then mixed and incubated.
- Control substances are immediately harvested and represent the concentration present at the start of the test, or time zero.
- At the conclusion of the contact time, a volume of the liquid test solution is harvested and chemically neutralized.
- Dilutions of the neutralized test solution are assayed using appropriate growth media to determine the surviving microorganisms at the respective contact times.
- Reductions of microorganisms are calculated by comparing initial microbial concentrations to final microbial concentrations.

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Criteria for Scientific Defensibility of an ASTM E2315 Study

For Microchem Laboratory to consider a Suspension Time Kill 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 10^6 cells/ml or greater.
2. Ordinary consistency between replicates must be observed for the time zero samples.
3. Positive/Growth controls must demonstrate growth of appropriate test microorganism.
4. Negative/Purity controls must demonstrate no growth of test microorganism.

Passing Criteria

ASTM International does not specify performance criteria, therefore it may be established by the Study Sponsor.

Testing Parameters used in this Study

Test Substance Volume:	1.00 g	Replicates:	Singlet
Control Substance Volume:	1.00 ml	Control Substance:	PBS

Culture Growth Media:	TSB & PDA	Culture Growth Time:	See Notes
Culture Dilution Media:	N/A	Inoculum Volume:	0.010 ml
Inoculum Concentration:	>1.0 x 10^6 CFU/ml	Contact Temp.:	Ambient (25°C ± 2°C)
Contact Time:	6 & 24 hours	Volume Harvested:	See Notes
Neutralizer (Vol.):	9.00 ml D/E + beads	Plating Media:	TSA & PDA
Enumeration Plate Incubation Temperature:	30°C ± 1°C & 36°C ± 1°C	Enumeration Plate Incubation Time:	24-48 hours

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Study Modifications and Notes

Test substance was provided as a viscous gel. 1.0 ± 0.5 gram of the test substance was weighed out into individual volumes per contact time per microorganism. Test substance was centrifuged at 1000 rpm for 2 minutes to collect at the bottom of the test vessel. 0.010 ml of the test microorganism culture was added to the test substance and manually mixed to dispense within the test substance. Inoculated test substances were held at room temperature for the duration of the contact time. At the close of each contact time, 9.0 ml of neutralization media (D/E broth) containing 10-15 sterile glass beads were added to the test substances. The neutralized test substances were vigorously vortex mixed, diluted, and assayed for surviving microorganisms.

Control Results

Neutralization Method: Verified | Media Sterility: Sterile
Growth Confirmation: Confirmed, Morphology on growth media

Calculations

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

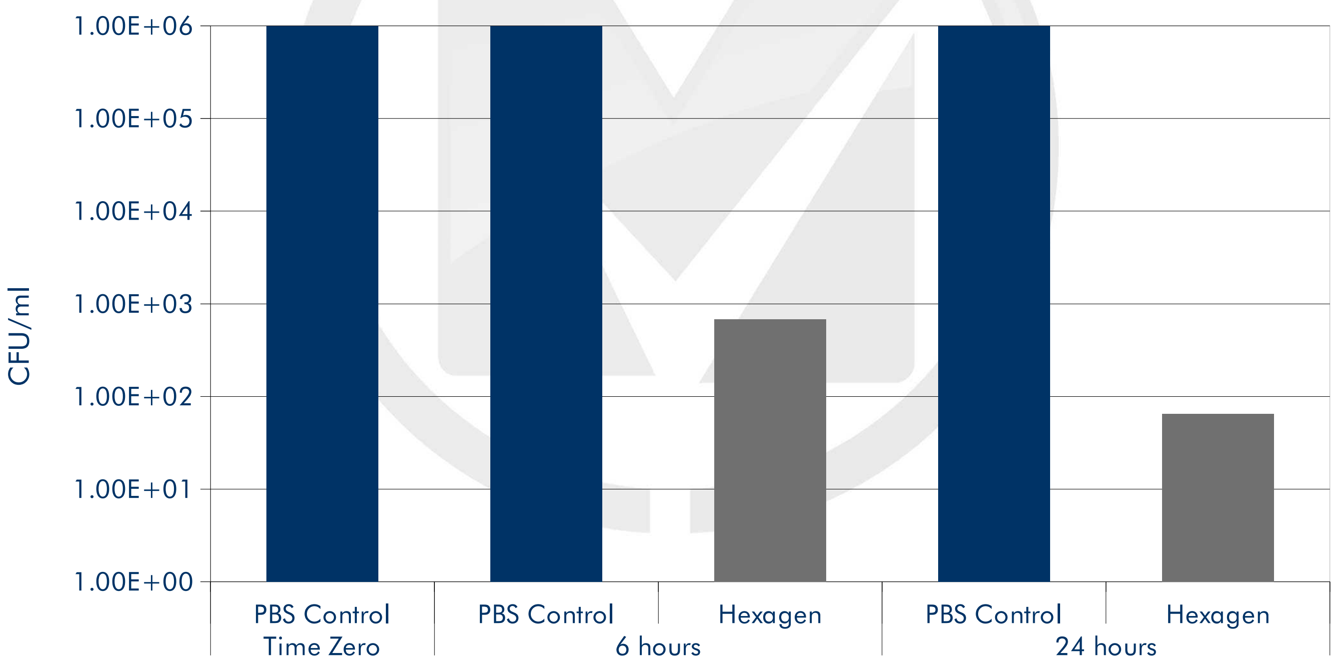
Where:
B = Number of viable test microorganisms in the control substance immediately after inoculation
A = Number of viable test microorganisms in the test substance after the contact time

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

Where:
B = Number of viable test microorganisms in the control substance immediately after inoculation
A = Number of viable test microorganisms in the test substance after the contact time

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

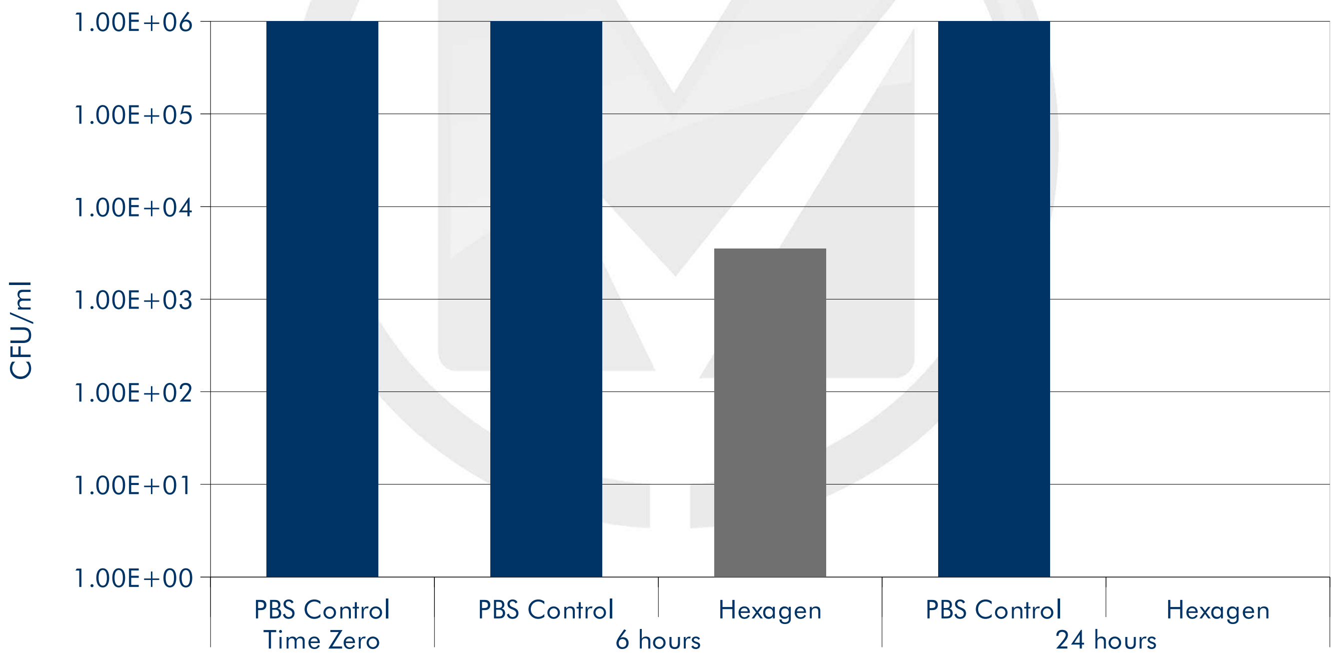
Test Microorganism	Contact Time	Test Substance	CFU/ml	Percent Reduction Compared to Parallel Control	Log_{10} Reduction Compared to Parallel Control
S. aureus 6538	Time Zero	PBS Control	2.70E+06	N/A	
	6 hours	PBS Control	1.00E+06		
		Hexagen	6.80E+02	99.93%	3.17
	24 hours	PBS Control	1.80E+06	N/A	
		Hexagen	6.50E+01	99.996%	4.44



RESULTS

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

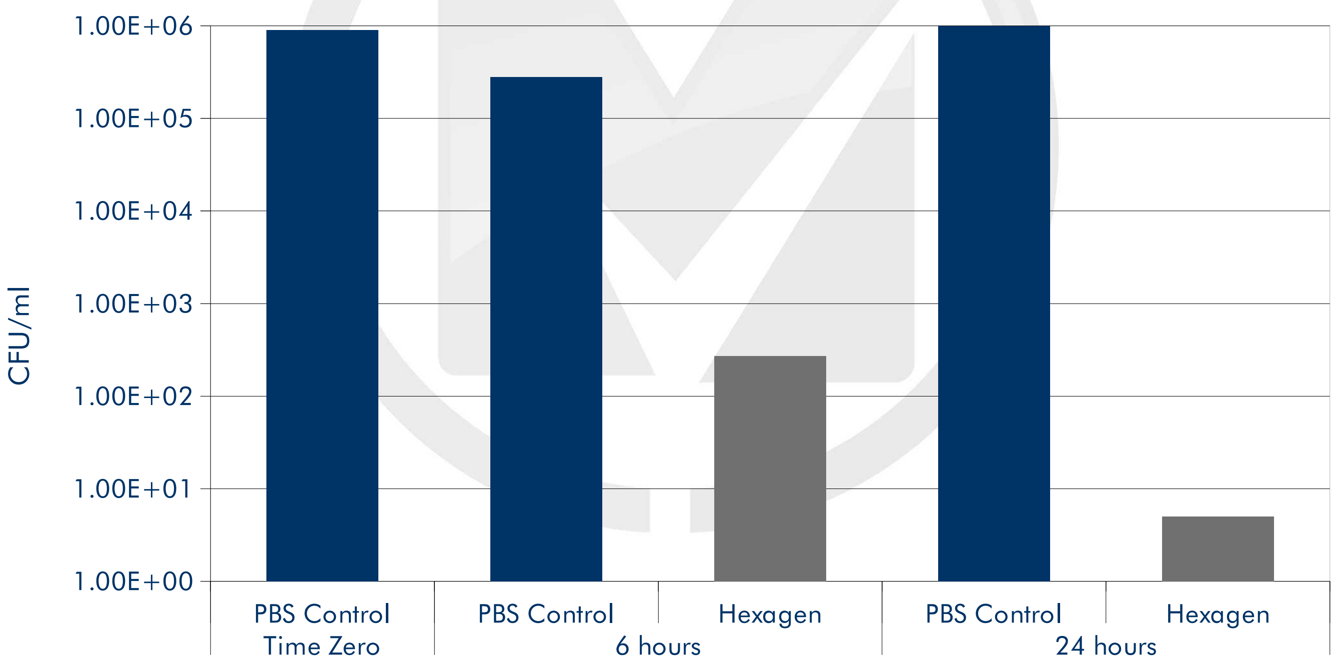
Test Microorganism	Contact Time	Test Substance	CFU/ml	Percent Reduction Compared to Parallel Control	Log_{10} Reduction Compared to Parallel Control
E. coli 8739	Time Zero	PBS Control	1.75E+06	N/A	
	6 hours	PBS Control	8.45E+06		
		Hexagen	3.50E+03	99.96%	3.38
	24 hours	PBS Control	3.20E+07	N/A	
		Hexagen	<5.00E+00	>99.99998%	>6.81



Note: The limit of detection is 5.00E+00 and is represented as <5.00E+00 in the table and as 0 in the graph.

Results of the Study- *P. aeruginosa* ATCC 9027

Test Microorganism	Contact Time	Test Substance	CFU/ml	Percent Reduction Compared to Parallel Control	Log_{10} Reduction Compared to Parallel Control
P. aeruginosa 9027	Time Zero	PBS Control	9.00E+05	N/A	
	6 hours	PBS Control	2.80E+05		
		Hexagen	2.70E+02	99.90%	3.02
	24 hours	PBS Control	2.94E+07	N/A	
		Hexagen	5.00E+00	99.99998%	6.77



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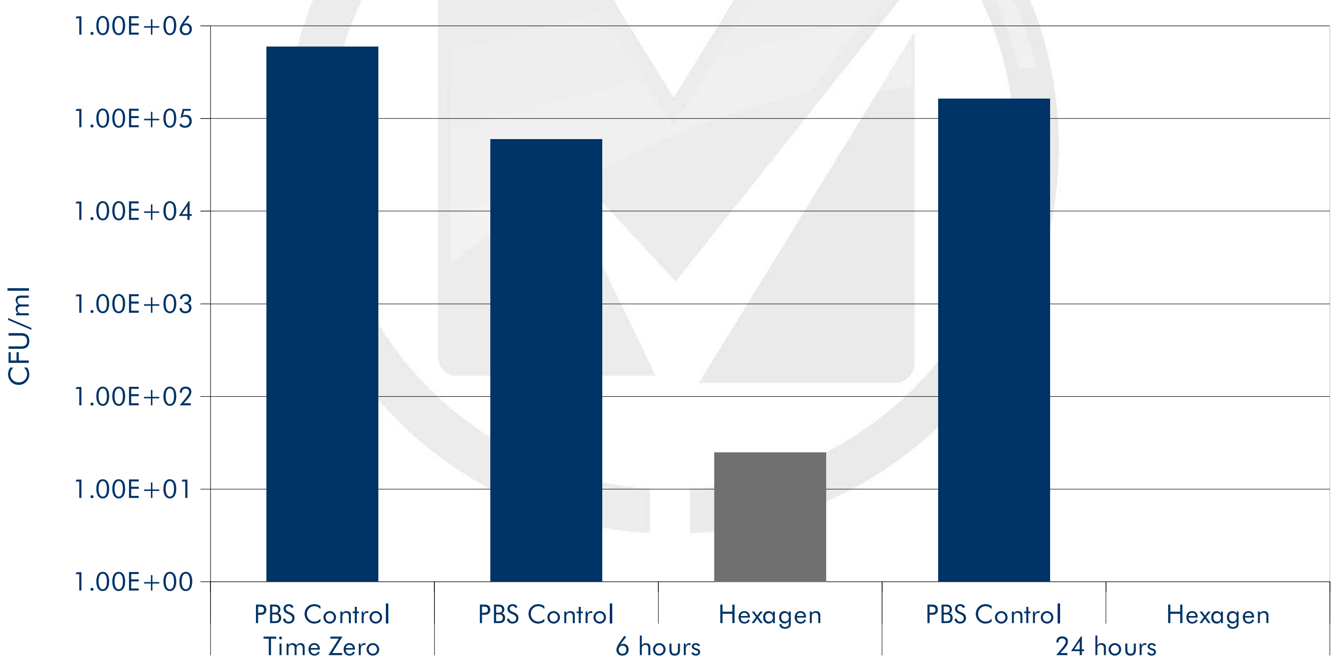
Results of the Study- *C. albicans* ATCC 10231

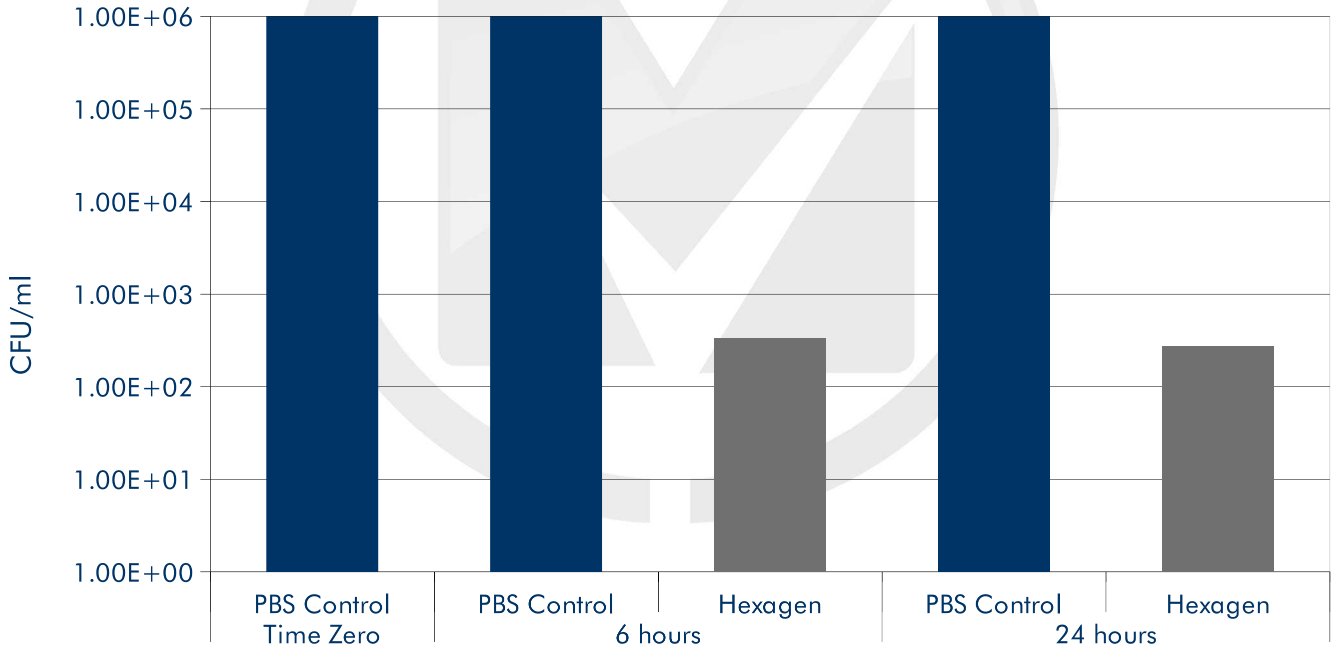
Test Microorganism	Contact Time	Test Substance	CFU/ml	Percent Reduction Compared to Parallel Control	Log_{10} Reduction Compared to Parallel Control
C. albicans 10231	Time Zero	PBS Control	5.95E+05	N/A	
	6 hours	PBS Control	6.00E+04		
		Hexagen	2.50E+01	99.958%	3.38
	24 hours	PBS Control	1.65E+05	N/A	
		Hexagen	<5.00E+00	>99.997%	>4.52



Note: The limit of detection is 5.00E+00 and is represented as <5.00E+00 in the table and as 0 in the graph.

Results of the Study- *S. aureus* ATCC 33591 (MRSA)

Test Microorganism	Contact Time	Test Substance	CFU/ml	Percent Reduction Compared to Parallel Control	Log_{10} Reduction Compared to Parallel Control
S. aureus 33591 (MRSA)	Time Zero	PBS Control	2.70E+06	N/A	
	6 hours	PBS Control	1.05E+06		
		Hexagen	3.35E+02	99.97%	3.50
	24 hours	PBS Control	1.65E+06	N/A	
		Hexagen	2.75E+02	99.98%	3.78



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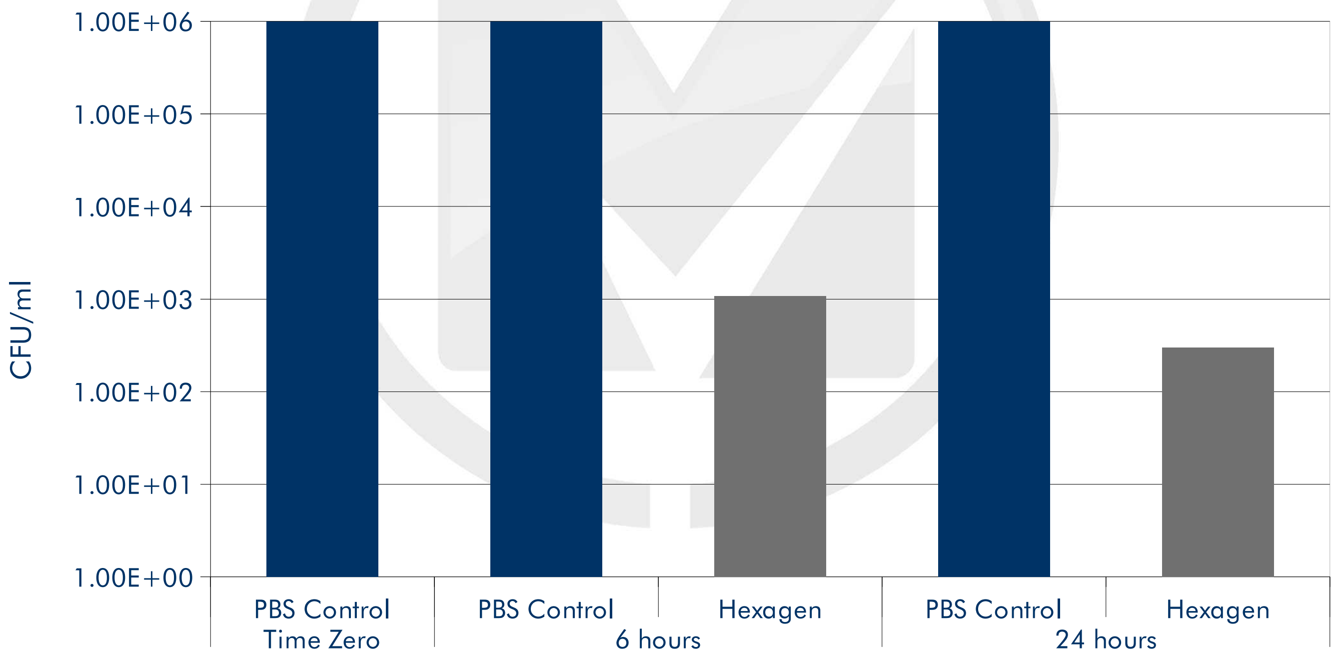
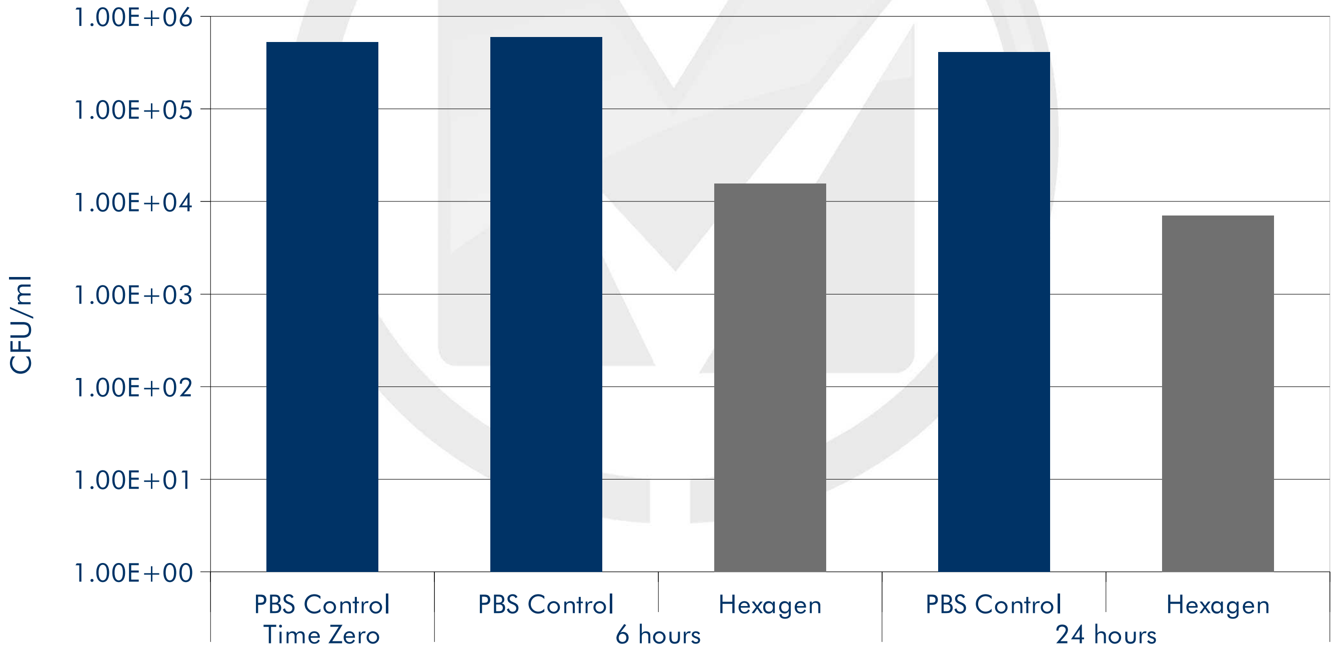
Results of the Study- *E. faecalis* ATCC 51299 (VRE)

Test Microorganism	Contact Time	Test Substance	CFU/ml	Percent Reduction Compared to Parallel Control	Log_{10} Reduction Compared to Parallel Control
E. faecalis 51299 (VRE)	Time Zero	PBS Control	1.40E+06	N/A	
	6 hours	PBS Control	1.30E+06		
		Hexagen	1.08E+03	99.92%	3.08
	24 hours	PBS Control	1.05E+06	N/A	
		Hexagen	3.00E+02	99.97%	3.54



Results of the Study- *A. niger* ATCC 9642

Test Microorganism	Contact Time	Test Substance	CFU/ml	Percent Reduction Compared to Parallel Control	Log_{10} Reduction Compared to Parallel Control
A. niger 9642	Time Zero	PBS Control	5.30E+05	N/A	
	6 hours	PBS Control	5.95E+05		
		Hexagen	1.55E+04	97.39%	1.58
	24 hours	PBS Control	4.10E+05	N/A	
		Hexagen	7.00E+03	98.29%	1.77



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