MICROCHEM LABORATORY

STUDY REPORT

Study Title

Antibacterial Activity and Efficacy of Preservative within GICC's Test Substance

Test Method

United States Pharmacopeial Convention Chapter 51
Antimicrobial Effectiveness Testing

Study Identification Number

NG13788

Study Sponsor

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Test Facility

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Your study was conducted by: Matthew South, B.S.

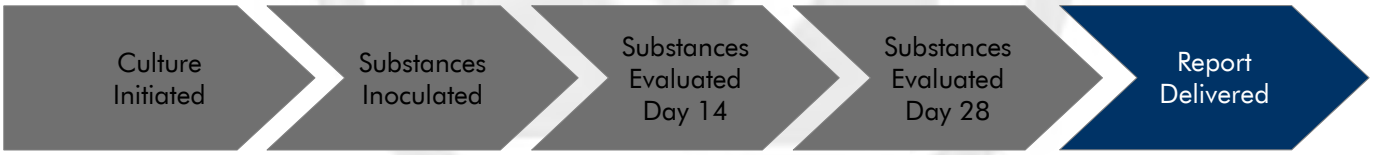
Laboratory Qualification Statement

Microchem 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. The laboratory also holds and ISO 17025 accreditation and undergoes annual audit for maintenance of this accreditation.

Laboratory Qualifications Specific to USP <51>

Microchem Laboratory has performed thousands of USP <51> tests on a broad array of test substances, against method specific and non-method specific bacterial and fungal species. The laboratory may also modify the USP <51> test as needed in order to accommodate customer needs. Every USP <51> test at Microchem Laboratory is performed in a manner appropriate to the test substances submitted by the Study Sponsor, while maintaining the integrity of the method.

Study Timeline



Bacteria 18SEP2019 **Yeast** 17SEP2019 **Mold** 06SEP2019	19SEP2019	03OCT2019	17OCT2019	24OCT2019

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Test Substance Information

The test substance was received on 11SEP2019.

Test Substance Received: Path-away/Protectence 2.0 (2% Concentration)

Test Substance arrived as ready-to-use.

Test Microorganism Information

The test microorganism(s) selected for this test:

Staphylococcus aureus 6538

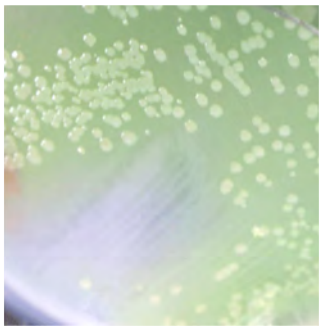
This bacterium is a Gram-positive, spherical-shaped, facultative anaerobe. *Staphylococcus* species are known to demonstrate resistance to antibiotics such as methicillin. *S. aureus* pathogenicity can range from commensal skin colonization to more severe diseases such as pneumonia and toxic shock syndrome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

Aspergillus brasiliensis 16404

This fungi is a conidiophore, or a sexual spore generating aerobic fungus. *A. brasiliensis*, formerly listed as a strain of *A. niger*, is related to other *Aspergillus* species in that they produce spores which are highly resistant to chemical and environmental conditions. *A. brasiliensis* is commonly used as a benchmark fungus for antimicrobial fungicides and preservatives used in pharmaceutical and personal care products.

Test Microorganism Information

The test microorganism(s) selected for this test:

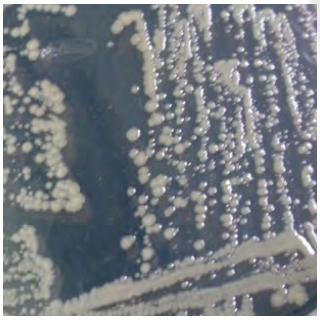


Pseudomonas aeruginosa 9027

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.

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 thrush. *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.



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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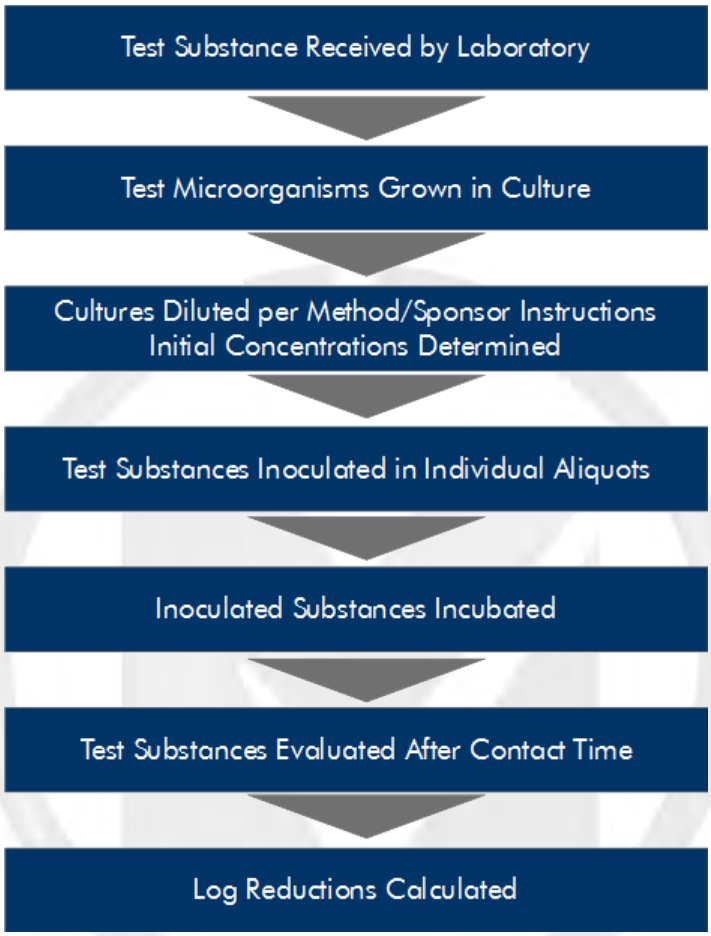
Diagram of the Procedure



Summary of the Procedure

- The test microorganisms are prepared by growth in liquid or on agar culture medium. Microorganisms grown in liquid culture are centrifuged and washed prior to test.
- Suspensions of test microorganisms are standardized by dilution in a buffered saline solution.
- Test and control substances are dispensed, in similar known volumes, to sterile vessels
- Independent volumes of Test and Control substances are inoculated with each test microorganism mixed and incubated. Control substances are immediately harvested and represent the concentration present at the start of the test, or time zero.
- Incubated Test Substances are harvested at the conclusion of each contact time by chemical neutralization.
- The number of surviving microorganisms at the respective contact times are assessed and logarithmic reductions are calculated based on initial concentrations observed at time zero.

Criteria for Scientific Defensibility of a USP <51> Study

For Microchem Laboratory to consider a USP <51> study to be scientifically defensible, the following criteria must be met:

1. The average number of viable test microorganisms recovered from the time zero samples must be approximately 1 x 10^5 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

Criteria for antimicrobial effectiveness is determined based the category to which a substances belongs. For Category 2 products, the criteria for bacteria is not less than 2-$\log_{10}$ from the initial count at 14 days, and no increase from the 14 day count at 28 days. The criteria for yeast and mold is no increase from the initial count at 14 and 28 days. No increase is defined as not more than 0.5 $\log_{10}$ higher than the previous value.

Testing Parameters

Test Substance Volume:	5 ml	Control Substance:	PBS (10 ml)
Replicates:	Single		

Culture Growth Media:	Tryptic Soy Broth (Bacteria) and Potato Dextrose Agar (Yeast & Fungi)		
Culture Growth Time:	18-24 hours (Bacteria), 48 hours (Yeast), 7 days (Fungi)		
Plating Media:	Tryptic Soy Agar (Bacteria) and Potato Dextrose Agar (Yeast & Fungi)		
Inoculum Concentration:	1.0 x 10^5 CFU/ml	Inoculum Volume:	0.025 ml
Observation Times:	14 and 28 Days	Volume Harvested:	0.100 ml
Enumeration Plate Incubation Temperature:	36.0°C±1°C (Bacteria), 30.0°C±2°C (Yeast and Fungi)		

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.

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Control Results

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

Calculations

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

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

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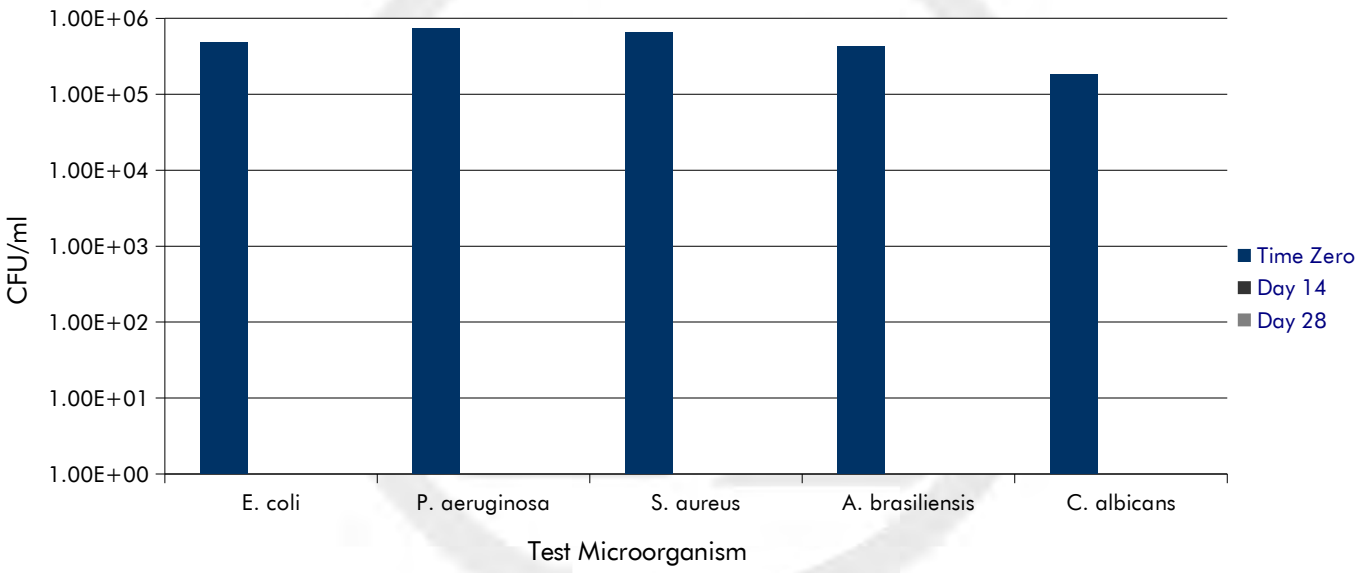
Results of the Study

Test Substance	Contact Time	Data Description	Test Microorganism				
			E. coli 8739	*P. aeruginosa* 9027	*S. aureus* 6538	*A. brasiliensis* 16404	*C. albicans* 10231
Path-away/ Protectence 2.0 (2% Concentration)	Time Zero	CFU/ml	4.80E+05	7.45E+05	6.55E+05	4.20E+05	1.85E+05
	Day 14	CFU/ml	<5.00E+01	<5.00E+01	<5.00E+01	<5.00E+01	<5.00E+01
		Log_{10} Reduction	>3.98	>4.17	>4.12	>3.92	>3.57
	Day 28	CFU/ml	<5.00E+01	<5.00E+01	<5.00E+01	<5.00E+01	<5.00E+01
		Log_{10} Reduction	>3.98	>4.17	>4.12	>3.92	>3.57



Note: The limit of detection for this assay was 50 CFU/ml. Values observed below this limit are presented as <5.00E+01 in the table and zero in the graph above.

RESULTS

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Results of the Study

Test Microorganism	Test Substance	Neutralization Validation Counts		Average NV Counts	Percent Recovery	Neutralization Scheme
E. coli ATCC 8739	Control	39	43	41	N/A	1:100 In Dey/Engley Broth
	Path-away/ Protectence 2.0 (2% Concentration)	38	44	41	100.00%	
P. aeruginosa ATCC 9027	Control	76	100	88	N/A	1:100 In Dey/Engley Broth
	Path-away/ Protectence 2.0 (2% Concentration)	93	85	89	101.14%	
S. aureus ATCC 6538	Control	66	59	62.5	N/A	1:100 In Dey/Engley Broth
	Path-away/ Protectence 2.0 (2% Concentration)	76	76	76	121.60%	
A. brasiliensis ATCC 16404	Control	47	45	46	N/A	1:100 In Dey/Engley Broth
	Path-away/ Protectence 2.0 (2% Concentration)	58	55	56.5	122.83%	
C. albicans ATCC 10231	Control	28	21	24.5	N/A	1:100 In Dey/Engley Broth
	Path-away/ Protectence 2.0 (2% Concentration)	18	28	23	93.88%	

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Study Conclusion

The product sample listed in the tables above was tested per Chapter USP <51> and successfully met the passing criteria per Chapter <51> of USP NF-2009.

The results of this study apply to the tested substance only. Extrapolation of findings to related materials is the responsibility of the Sponsor.