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USP Antimicrobial Preservative Effectiveness Test GLP Report

Test Article:	Batch #SSG241017
Purchase Order:	1728
Study Number:	1006373-S01
Study Received Date:	30 Nov 2017
Testing Facility:	Nelson Laboratories, LLC, a Business Unit of Sterigenics International 6280 S. Redwood Rd. Salt Lake City, UT 84123 U.S.A.
Test Procedure(s):	Standard Test Protocol (STP) Number: STP0131 Rev 05 Customer Specification Sheet (CSS) Number: 201707525 Rev 01
Deviation(s):	None

Summary: This study was performed to determine the survival rate of various organisms in the test product. The test employed methods designed to determine antimicrobial effectiveness described in the United States Pharmacopeia (USP). All test method acceptance criteria were met. Interpretation of the data is the responsibility of the sponsor and no conclusion can be made by Nelson Laboratories, LLC (NL).

Test articles of the product were inoculated with five standard test organisms. The inoculated test articles were then stored for a total of 28 days at 20-25°C. Aliquots from the test articles were removed and assayed for surviving organisms at 0 hour, 7 day, 14 day, and 28 day time intervals to satisfy the USP testing requirements. The log reduction in the level of the test organisms was calculated for each time interval.

The suitability testing showed adequate suitability of all products tested when challenged against the standard test organisms.

Results: The greater than (>) values represent the detectable limits of the test where zero colony forming units (CFU) were observed on the plates. The approximate (~) symbol is applied to results where plate counts fell outside of the statistically accurate range of 25-250 CFU for bacteria and yeast and 8-80 CFU for mold.

ilac-MRA ANAB ACCREDITED TESTING LABORATORY

Study Director — Danielle Greening

23 Feb 2018
Study Completion Date

1006373-S01

jhs FRT0131-0001 Rev 5

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Inoculum Control:

Organism	Challenge Titer at 0 Hour (CFU/mL)
Aspergillus brasiliensis	3.9×10^5
Candida albicans	3.2×10^5
Escherichia coli	3.8×10^5
Staphylococcus aureus	1.3×10^5
Pseudomonas aeruginosa	2.5×10^5

Summary of Log Reduction:

Organism	Time Interval			
	0 Hour	7 Day	14 Day	28 Day
A. brasiliensis	-0.13	~4.17	>4.29	>4.29
C. albicans	>4.21	>4.21	>4.21	>4.21
E. coli	~4.05	>4.27	>4.27	>4.27
S. aureus	0.24	>3.81	>3.81	>3.81
P. aeruginosa	>4.10	>4.10	>4.10	>4.10

Suitability Data:

Organism	Control	Test Article	Percent of Control (%)
A. brasiliensis	27	29	107
C. albicans	50	47	94
E. coli	52	54	104
S. aureus	35	34	97
P. aeruginosa	53	60	113

Test Method Acceptance Criteria: Positive controls are positive for growth of the indicator organisms. Negative controls are negative for growth of the indicator organisms. The suitability control must demonstrate a ≥50% organism recovery at the reported product dilution.

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Interpretation of Results:

USP Categories: According to USP, products are placed into categories according to product type. The product category will determine the acceptance criteria for the test. The following table provides a description of the current USP categories.

Category	Product Description
1	Injections, other parenterals including emulsions, otic products, sterile nasal products, and ophthalmic products made with aqueous bases or vehicles.
2	Topically used products made with aqueous bases or vehicles, non-sterile nasal products, and emulsions, including those applied to mucous membranes.
3	Oral products other than antacids, made with aqueous bases or vehicles.
4	Antacids made with an aqueous base.

USP Antimicrobial Effectiveness Acceptance Criteria:

No increase is defined as not more than a 0.5 $\log_{10}$ unit higher than the previous value measured.

Category 1 Products	
Bacteria:	Not less than 1.0 log reduction from the initial calculated count at 7 days, not less than 3.0 log reduction from the initial count at 14 days, and no increase from the 14 days' count at 28 days.
Yeast & Molds:	No increase from the initial calculated count at 7, 14 and 28 days.
Category 2 Products	
Bacteria:	Not less than 2.0 log reduction from the initial count at 14 days, and no increase from the 14 days' count at 28 days.
Yeast & Molds:	No increase from the initial calculated count at 14 and 28 days.
Category 3 Products	
Bacteria:	Not less than 1.0 log reduction from the initial count at 14 days, and no increase from the 14 days' count at 28 days.
Yeast & Molds:	No increase from the initial calculated count at 14 and 28 days.
Category 4 Products	
Bacteria, Yeast, & Molds:	No increase from the initial calculated count at 14 and 28 days.

Procedure: The following organisms were tested:

1) *Aspergillus brasiliensis* ATCC #16404
   [Mold]
2) *Candida albicans* ATCC #10231
   [Yeast]
3) *Escherichia coli* ATCC #8739
   [Bacteria, Gram (-) bacillus]
4) *Staphylococcus aureus* ATCC #6538
   [Bacteria, Gram (+) cocci]
5) *Pseudomonas aeruginosa* ATCC #9027
   [Bacteria, Gram (-) bacillus]

The bacteria were transferred to Mueller Hinton Broth and incubated at 30-35°C for 18-24 hours. The *C. albicans* and *A. brasiliensis* were transferred to Sabouraud dextrose agar (SDEX). The *C. albicans* was incubated at 20-25°C for 44-52 hours and the *A. brasiliensis* was incubated at 20-25°C for 6-10 days.

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A. brasiliensis and *C. albicans* were harvested using Sterile Pure Water (PURW). The *A. brasiliensis* was filtered through sterile glass wool, or another appropriate filtration method, and all cultures were vortexed to break up clumps. Centrifugation was performed on the cultures after harvest at 3500-4000 x g (5400-5800 rpm) for approximately 10 minutes. The supernatant was removed and the culture was re-suspended with equal volumes of appropriate media. Each suspension was adjusted to approximately 10^8 CFU/mL. The actual concentration of each culture was determined using the positive control values.

These results represent a single analysis plated in triplicate. Individual test articles of the test product were prepared for each challenge organism. The tubes containing the test article were inoculated with the test organisms using a calibrated micropipettor. The volume of the inoculum was between 0.5% and 1.0% of the volume of the product. The initial concentrations of the test organisms within the test articles were approximately 10^5-10^6 CFU/mL. The test articles were well mixed.

Positive control tubes were prepared for each organism using Purified Water (PURW). The volume used for the positive control was equivalent to that used in the test articles. Negative controls were also prepared. The positive controls were then inoculated in the same manner as the test articles. All test articles were stored at 20-25°C for a total of 28 days.

Control tubes and test articles were assayed immediately to determine the initial concentration of organisms in each tube. The test articles were assayed at the following intervals: 0 hour, 7 day, 14 day, and 28 day.

Test article aliquots at each interval were diluted in Dey-Engley Neutralizer Broth (DEYE) and plated on TSA or equivalent media for bacteria and SDEX for *C. albicans* and *A. brasiliensis*. The bacteria plates were incubated at 30-35°C for 3-5 days. The *C. albicans* plates were incubated at 20-25°C for 3-5 days and the *A. brasiliensis* plates were incubated at 20-25°C for 3-7 days.

Additional controls were performed to ensure suitability was achieved during testing. This was performed by making a 1:10 ratio of test article to DEYE. This simulates the highest concentrations of the product plated, and represents the worst case for suitability. The tubes were inoculated with organism suspensions to reach final concentrations of <250 CFU/plate on the recovery plates. Control tubes of DEYE, using the same volume as the neutralizer and test article mix, were inoculated concurrently with the same cultures and plated. Suitability is demonstrated when the number of colonies on the test article plates demonstrates at least 50% recovery when compared to the control plates.

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Quality Assurance Statement

Compliance Statement: The test was conducted in accordance with the USFDA (21 CFR Parts 58, 210, 211, and 820) Regulations. This final report reflects the raw data.

Activity	Date
Study Initiation	11 Jan 2018
Phase Inspected by Quality Assurance: Plate Counts	20 Feb 2018
Audit Results Reported to Study Director	20 Feb 2018
Audit Results Reported to Management	21 Feb 2018

Scientists	Title
Thomas Pace	Supervisor
Danielle Greening	Study Director

Data Disposition: The study plan, raw data and final report from this study are archived at Nelson Laboratories, LLC or an approved off-site location.

Quality Assurance

22 Feb 2018
Date

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