

November 29, 2018

FIRST FISHERY DEVELOPMENT SERVICES INC.
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Anresco No. 1020180210
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Sample information

Product	Olive Leaf Nasal Spray Challenge Study
Sampling	Received from Client.
Received	October 01, 2018
Analyses Date	October 1, 2018 to November 22, 2018

The Seagate Olive Leaf Nasal Spray was submitted to determine the effectiveness of the antimicrobial agent. Prior to inoculating the samples with known organisms, the initial microbial load of the nasal spray was determined. The Olive Leaf Nasal Spray did not have any viable organisms present. The sample was then subjected to the P/E Challenge Study.

SAMPLE PREPARATION

UPS Method <51>, E. coli ATCC 25922, Ps. aeruginosa ATCC 10145, Staph. aureus ATCC 25923, Saccharomyces cerevisiae ATCC 9080 and Aspergillus niger ATCC16888 will be inoculated to the Olive Leaf Nasal Spray to yield approximately 1×10^5 to 1×10^6 organisms per ml of sample. These were the available organisms for the study. The ATCC strains differ from the ones listed in UPS <51>. Candida albicans was not available at the time of the study but Saccharomyces was available and used in its place.

0.3 ml of each inoculum was aseptically added to 30 ml of sample and immediately shaken to evenly distribute the organism in solution. For Time 0, 1 Min. and 24 Hr, the samples were plated at 1:100, 1:1000 and 1:10,000 dilution in anticipation of the survival of the designated organisms. In addition, a 30 ml of sterile saline was also inoculated to run in parallel with the sample. This will determine the viability of each organism throughout the study.

The inoculated sample was plated again at Day 7, Day 14, Day 21 and Day 28. The results are listed in Table I - Table III on the following pages.

OBSERVATIONS

All organisms were not detected at Time 0 reported as <1,000. The samples were plated to anticipate a high number of organisms. The starting dilution was 1/1000. In the following weeks, the lowest tested dilution was 1/10. Aspergillus and Saccharomyces was detected at very low levels at Time 0 and they were not detected at 24 Hours. The saline blank for each organism was within the expected range of 1×10^5 and 1×10^6 organisms per ml. The Saccharomyces and Aspergillus was repeated with higher inoculum and plated. The initial inoculated count was in the 10^2 and not detectable by at 24 Hr and 7 Day.

CONCLUSION

The submitted sample met the requirements for antimicrobial effectiveness as stated in USP 24 <51>. The criteria is as follows:

Bacteria (E. coli, Pseudomonas and Staph) - Not less than a 1 log reduction from the initial calculated count at Day 7 and not less than 3.0 log reduction from the initial count at Day 14 and no increase from Day 14 to Day 28.
Yeast/Mold (Saccharomyces & Aspergillus) - No increase from the initial calculated count at Day 7, Day 14, and Day 28.

The results are listed on the following pages.

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Table I - Media

MEDIA	Manufacturer	Lot No.	Expiration
Chromagar Pseudomonas	DRG International	P001109	2019.07
CHromagar Staph	DRG International	P001282	2020.01
Potato Dextrose Agar	EMD	VM814730805	1-13-19
Hardychrom ECC	Hardy Diagnostics	421577	5-10-19

Table II - Challenge Organisms

Organisms	ATCC No.	Inoculum CFU/ml	Estimated Initial Sample Concentration CFU/ml
E. coli	25922	1.2×10^7	1.2×10^6
P. aeruginosa	10145	4.0×10^8	4.0×10^6
Staph aureus	25923	3.9×10^8	3.9×10^6
Aspergillus niger	16888	1.0×10^5	1.0×10^3
Saccharomyces	9080	5.3×10^6	5.3×10^4
<i>0.3 ml of each inoculum to each 30ml sample</i>			
<i>ATCC = American Type Culture Collection</i>			
<i>CFU = Colony Forming Units</i>			

Table III - INHIBITION SCREEN

	DILUTIONS			
	1:1	1:10	1:100	1:1000
E. coli	Growth	Growth	Growth	Growth
Pseudomonas	Growth	Growth	Growth	Growth
Staph	Growth	Growth	Growth	Growth
Saccharomyces	Growth	Growth	Growth	Growth
Aspergillus	Growth	Growth	Growth	Growth
<i>Samples were plated with 1 ml of each of the dilutions. Agars were seed with each organisms and poured to each sample. Reactions were recorded base on Growth and No Growth</i>				

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Table IV - Microbial Load over 28 Days

	Time 0	60 minute	24 Hr	Day 7	Day 14	Day 21	Day 28
E. COLI							
Nasal Spray	<10,000	<10,000	<10	<10	<10	<10	<10
Saline	1.4×10^7	*	*	1.3×10^7	$3/0 \times 10^7$	2.7×10^7	2.9×10^7
STAPH							
Nasal Spray	<10,000	<10,000	<10	<10	<10	<10	<10
Saline	5.5×10^6	*	*	3.0×10^6	3.2×10^6	5.0×10^6	2.5×10^6
PSEUDOMONAS							
Nasal Spray	<10,000	<10,000	<10	<10	<10	<10	<10
Saline	9.1×10^6	*	*	7.4×10^6	3.6×10^6	1.4×10^6	2.0×10^6
SACCHAROMYCES							
Nasal Spray	<10,000	<10,000	<10	<10	<10	<10	<10
Saline	4.3×10^4	*	*	1.2×10^4	2.1×10^3	4.0×10^3	3.4×10^3
ASPERGILLUS							
Nasal Spray	<10,000	<10,000	<10	<10	<10	<10	<10
Saline	400	*	*	600	40	1,000	3,200
* Not Performed							

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