NELSON
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FINAL REPORT

USP MICROBIAL LIMITS

PROCEDURE NO. STP0041 REV 03
PROTOCOL DETAIL SHEET NO. 200803325 REV 01

LABORATORY NO. 452814

PREPARED FOR:

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QAU AUDIT STATEMENT

[X] USFDA (21 CFR PART 58) [ ] USEPA (40 CFR PART 160)

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1. The test was conducted in accordance with the USFDA or USEPA Regulations as noted above.

2. In accordance with the Good Laboratory Practice Regulations, the Fungal Plate Count phase of this study was inspected by the Quality Assurance Unit on: 16 Dec 2008. The findings of the inspection(s) were reported to the Study Director on: 19 Dec 2008 and to Management on: 20 Dec 2008.

3. The Quality Assurance Unit has reviewed this report and has determined that the methods and standard testing procedures are accurately described, and that the reported results accurately reflect the raw data.

4. The name of the study director, the names of other scientists or professionals, and the names of all supervisory personnel, involved in the study:

Sara Toole
Jonathon Swenson
Dr. Jerry Nelson
Jeff Hills

QUALITY ASSURANCE: Michelle Smith DATE: 22 Dec 2008

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USP MICROBIAL LIMITS

LABORATORY NUMBER:	452814
PROCEDURE NUMBER:	STP0041 REV 03
PROTOCOL DETAIL SHEET NUMBER:	200803325 REV 01
SAMPLE SOURCE:	EnzySurge
SAMPLE IDENTIFICATION:	Refer to Tables 1 and 2 P.O. #1008
DEVIATIONS:	None
AMOUNT OF SAMPLE TESTED:	
Qualification (FCDM and ALBR)	10 mL per organism
Plate Count (FCDM)	10 mL
Selective Screening (FCDM and ALBR)	10 mL per diluent
PLATE COUNT TEST DILUTION:	1:10
SELECTIVE SCREENING TEST DILUTION:	1:10
QUALIFICATION REFERENCE LAB #:	Concurrent
PROTOCOL APPROVAL DATE:	10 Dec 2008
SAMPLE RECEIVED DATE:	26 Nov 2008
LAB PHASE START DATE:	10 Dec 2008
LAB PHASE COMPLETION DATE:	20 Dec 2008
REPORT ISSUE DATE:	22 Dec 2008

INTRODUCTION:

This procedure is designed to determine the presence of *Staphyloccus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa, Salmonella* sp. and other related organisms that may be objectionable or considered pathogenic in a non-sterile sample. The presence of these organisms indicates an environment that allows growth of similar pathogenic bacteria.

ACCEPTANCE CRITERIA:

Raw plate count results should be within 30-300 colony forming units (CFU) per plate or reported as an estimate. *Aspergillus niger* and other similar organisms can accurately be read with only 8-80 CFU per plate. If no colonies are found, the results will be reported as less than the sample dilution. The plate count is valid when the negative monitors are within the parameters established in the current procedure. Positive controls for qualification should demonstrate characteristic growth. Negative test monitors for the selective screening should not demonstrate growth of the indicator organism.

PROCEDURE:

Sample Preparation: The sample was prepared by combining 10 mL of the sample with 90 mL of 50 mM Tris-buffer pH 7.45. Ten milliliter aliquots of this solution was then placed in 90 mL of fluid casein digest-soy lecithin-polysorbate 20 medium (FCDM) and acumedia lactose broth (ALBR).

Plate Counts: Using the pour plate technique, 1 mL of the sample was plated in soybean casein digest agar (SCDA) in triplicate for bacterial counts. The same procedure was performed using potato dextrose agar (PDXA) in triplicate for fungal counts. The SCDA plates were incubated for 48-72 hours at 30-35°C while the PDXA plates were incubated for 5-7 days at 20-25°C.

Sample Enrichment: The samples were diluted in FCDM for *Staphylococcus aureus* and *Pseudomonas aeruginosa* screening, and ALBR for *Salmonella* and *Escherichia coli* screening. The enrichment broths were allowed to incubate for 24-48 hours at 30-35°C.

Microbial Screening: Following enrichment incubation, the broths were transferred or streaked to the appropriate media for incubation as follows:

Salmonella:	Selenite-cystine broth and Tetrathionate broth - 12-24 hours at 30-35°C Brilliant green, Bismuth sulfite, and XLD agars - 24-48 hours at 30-35°C
P. aeruginosa:	Cetrimide agar - 24-48 hours at 30-35°C
S. aureus:	Mannitol salt agar - 24-48 hours at 30-35°C
E. coli:	MacConkey agar - 24-48 hours at 30-35°C

Any suspect colonies were verified using biochemical tests.

QUALIFICATION:

Qualification testing was performed on the initial test of this sample type as follows:

Twenty-four hour broth cultures of the four test organisms were grown at 30-35°C and diluted 1:1000. The organism aliquot used for inoculation was ≤ 1% of the sample preparation. The inoculum was added within one hour of the dilution of the sample in the broths. The screening procedure was followed. All four test organisms must be recovered, demonstrating neutralization of the sample.

RESULTS:

SilverStream Lot #SIL-001271008, Silver Buffered Solution 250mL, Active Ingredient: Each mL contains Silver Nitrate 0.1mg was tested and passed qualification at the 1:10 dilution. Routine analysis may be performed at the 1:10 sample dilution.

The total aerobic and fungal counts are summarized in Table 1. The plate count results are not qualified for bacterial or fungal recovery.

Screening results for the USP microbial limits method are summarized in Table 2.

Testing met the acceptance criteria previously stated in this report.

CONCLUSION:

Interpretation of the data is the responsibility of the sponsor and no conclusion can be made by Nelson Laboratories, Inc. (NLI).

DATA DISPOSITION:

The raw data and final report from this study are archived at NLI or an approved off-site location.

STATEMENT OF UNCERTAINTY:

If applicable, the statement of uncertainty is available to sponsors upon request.

Jonathan Swenson, B.S.
Study Director

22-Dec-08
Study Completion Date

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EnzySurge
Lab Number 452814

USP Microbial Limits

TABLE 1. Total Aerobic and Fungal Counts
(CFU per mL)

SAMPLE IDENTIFICATION	TOTAL AEROBIC MICROBIAL COUNT	COMBINED MOLD AND YEASTS COUNT
SilverStream Lot #SIL-001271008, Silver Buffered Solution 250mL, Active Ingredient: Each mL contains Silver Nitrate 0.1mg	<10	<10

<10 = None detected

TABLE 2. Pathogen Screening Results
Sample Identification: SilverStream Lot #SIL-001271008, Silver Buffered Solution 250mL, Active Ingredient: Each mL contains Silver Nitrate 0.1mg

ORGANISM	RESULTS	
Staphylococcus aureus	[ ] PRESENCE	[X] ABSENCE
Pseudomonas aeruginosa	[ ] PRESENCE	[X] ABSENCE
Escherichia coli	[ ] PRESENCE	[X] ABSENCE
Salmonella	[ ] PRESENCE	[X] ABSENCE