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FINAL REPORT

QUANTITATIVE MINI KILL TIME TEST

PROTOCOL NO. 200308406-03

LABORATORY NO. 233010.1 AMENDED

PREPARED FOR:

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STUDY DIRECTOR GLP CERTIFICATION

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

QUANTITATIVE MINI KILL TIME TEST

I CERTIFY THAT THE TEST WAS CONDUCTED IN ACCORDANCE

WITH THE USFDA OR USEPA REGULATIONS AS NOTED ABOVE.

LABORATORY NO. 233010.1 AMENDED

STUDY DIRECTOR: _____ DATE: 31 Oct 2003

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QAU AUDIT STATEMENT

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

QUANTITATIVE MINI KILL TIME TEST

Study Director: Final Report Dated:

Emily F. Mitzel, B.S., M.S. 22 Jul 2003

1. The test was conducted in accordance with the USFDA or USEPA Regulations as noted above. All laboratory results pertaining to this study are recorded in Nelson Laboratories' Data File Number 233010.1 Amended.

2. In accordance with the Good Laboratory Practice Regulations, the Plating 5 day phase(s) of this study was inspected by the Quality Assurance Unit on: 28 Apr 2003. The findings of the inspection(s) were reported to Management and to the Study Director on: 28 Apr 2003.

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

QUALITY ASSURANCE: _____ DATE: 31 Oct 2003

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QUANTITATIVE MINI KILL TIME TEST

LABORATORY NUMBER:	233010.1 Amended
PROTOCOL NUMBER:	200308406-03
SAMPLE SOURCE:	SinoFresh Laboratories, Inc.
SAMPLE IDENTIFICATION:	Pilot Sino Nasal Lot #0017
DEVIATIONS:	None
DATA ARCHIVE LOCATION:	Sequentially by lab number
PROTOCOL APPROVAL DATE:	08 Apr 2003
SAMPLE RECEIVED DATE:	08 Apr 2003
LAB PHASE START DATE:	08 Apr 2003
LAB PHASE COMPLETION DATE:	21 Jul 2003
REPORT ISSUE DATE:	22 Jul 2003
STUDY COMPLETION DATE:	28 Jul 2003
AMENDED REPORT ISSUE DATE:	30 Oct 2003
TOTAL NUMBER OF PAGES:	11

AMENDMENT JUSTIFICATION:

At the sponsor's request, a reference was removed from the report.

REFERENCE:

United States Pharmacopeia & National Formulary. Current Revision. Antimicrobial Effectiveness, p. 1809-1811. United States Pharmacopeial Convention, Inc., Rockville, MD.

Block, Seymour S. 1991. Disinfection, Sterilization and Preservation. 4th Ed. Philadelphia: Lea & Febiger. Chapter 57.

INTRODUCTION:

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).

The samples of the product were inoculated, in duplicates, with 14 test organisms. The inoculated samples were then incubated for a total of 7 days at 20-25°C. Aliquots from the samples were immediately removed and assayed for surviving organisms at 0 hour, 6 hour, 24 hour, 72 hour, 5 day, and 7 day time intervals. The log reduction in the level of the test organisms was calculated for each time interval.

PROCEDURE:

The following organisms were tested:

1) *Staphylococcus aureus* ATCC #6538

2) *Pseudomonas aeruginosa* ATCC #9027

3) *Escherichia coli* ATCC #8739

4) *Streptococcus pyogenes* ATCC #8669

5) *Stachybotrys chartarum* ATCC #9182

6) *Alternaria alternata* ATCC #44501

7) *Cladosporium herbarum* ATCC #28987

8) *Penicillium funiculosum* ATCC #10509

9) *Candida albicans* ATCC #10231

10) *Fusarium solani* ATCC #36031

11) *Aspergillus niger* ATCC #16404

12) *Haemophilus influenzae* ATCC #9795

13) *Moraxella (Branhamella) cattarrhalis* ATCC #25238

14) *Staphylococcus aureus* ATCC #700698

Staphylococcus aureus ATCC #6538, *Pseudomonas aeruginosa, Escherichia coli, Streptococcus pyogenes,* and *Staphylococcus aureus* ATCC #700698 were transferred to soybean casein digest agar (SCDA) and incubated at 35-39°C for 18-24 hours. *Moraxella (Branhamella) cattarrhalis* was transferred to SCDA and incubated at 30-35°C for 44-52 hours. *Stachybotrys chartarum* and *Cladosporium herbarum* were transferred to Malt Extract agar (MEXA) and incubated at 20-25°C for 6-10 days. *Candida albicans, Alternaria alternata, Penicillium funiculosum, Aspergillus niger,* and *Fusarium solani* were transferred to Sabouraud dextrose agar (SDEX). *C. albicans* was incubated at 20-25°C for 44-52 hours and the other molds were incubated at 20-25°C for 6-10 days. *Haemophilus influenzae* was transferred to Peptic Digest Agar (PDA) and was incubated at 30-35°C for 44-52 hours.

The bacteria and *C. albicans* were harvested using 0.9% saline, *A. niger* was harvested with 0.9% saline containing 0.05% polysorbate 80 (Tween®), and the additional molds were harvested with 0.9% saline containing 0.05% polysorbate 80 (Tween®) and macerated in tissue grinders. The mold cultures were filtered through gauze and vortexed vigorously to break up clumps.

The titer of the each bacteria was adjusted to approximately 10^8 colony forming units (CFU) per mL using visual turbidity. The concentration of the molds were not adjusted. The actual titer of each culture was determined using the positive control values.

Two 7.0 mL samples of the test product were prepared for each challenge organism. The duplicate tubes containing 7.0 mL were then inoculated with 70 μL of the test organism using a calibrated micropipettor. The volume of the inoculum was between 0.5% and 1.0% of the volume of the product. The final concentration of the test preparation was approximately 10^5 and 10^6 CFU/mL of product. The samples were well mixed.

Two positive control tubes were prepared for each organism using sterile water. Negative controls were also prepared. A 70 μL aliquot of the test organism was added to 7 mL of sterile water for the positive control. All test samples were stored at 20-25°C for a total of 7 days.

Control tubes and test vials were assayed immediately to determine the initial concentration of organisms in each tube. The test suspensions were assayed at the following intervals: Time 0, 6 hour, 24 hour, 72 hour, 5 days, and 7 days.

Sample aliquots at each interval were diluted in letheen broth (LETH) and plated on SCDA and PDA for bacteria and SDEX and MEXA for the molds. 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 mold plates were incubated at 20-25°C for 3-7 days.

Additional controls were performed to ensure neutralization. This was performed by adding 1.0 mL of un-inoculated product to 9.0 mL of LETH blanks and then performing two 1/10 dilutions of the un-inoculated product. This simulates the highest concentrations of the product tested, and represents the worst case for neutralization. These tubes were then inoculated with 1.0 mL of a 100-1000 CFU/mL organism suspension, and plated in 0.5 mL aliquots on the appropriate agar. Control blanks of LETH were inoculated concurrently with the same known cultures and plated. Neutralization is demonstrated when the number of colonies on the plates with test product added does not vary significantly from the number on the control plates.

RESULTS:

The results for the samples are reported in Tables 1 and 2. All values are shown in CFU/mL. The less than (<) values represent the detectable limits of the test where zero CFU were observed on the plates.

The neutralization data is found in Table 3. The neutralization testing showed growth of the test organisms comparable to the known number of organisms added to the neutralized test product. This indicates effective neutralization of the sample. *Staphylococcus aureus* ATCC #700698, *Alternaria alternata, Pseudomonas aeruginosa, Stachybotrys chartarum, Cladosporium herbarum, and Candida albicans* all neutralized at a 1/10 dilution. *Staphylococcus aureus* ATCC #6538, *Moraxella (Branhamella) cattarrhalis, Streptococcus pyogenes, Penicillium funiculosum, Fusarium solani, Escherichia coli, Haemophilus influenzae*, and *Aspergillus niger* and neutralized at 1/100 dilution.

CONCLUSION:

The log reduction results for Pilot Sino Nasal Lot #0017 product (full strength) were greater than: 3.83 for *Staphylococcus aureus* #6538, 4.07 for *Pseudomonas aeruginosa*, 3.74 for *Escherichia coli*, 4.83 for *Candida albicans*, 3.33 for *Streptococcus pyogenes*, 1.25 for *Alternaria alternata*, 1.30 for *Cladosporium herbarium*, 1.61 for *Penicillium funiculosum*, 2.03 for *Fusarium solani*, 2.07 for *Haemophilus influenzae*, 2.79 for *Moraxella cattarrhalis*, 4.30 for *Staphylococcus aureus* #700698, 3.49 for *Aspergillus niger*, and 2.76 for *Stachybotrys chartarum.*

Quality Assurance Reviewer

Emily F. Mitzel, B.S., M.S.
Study Director

31 Oct 2003
Amended Report Date

mjm

TABLE 1. Summary of Results
0 hour, 6 hour, 24 hour, 72 hour, 5 day, and 7 day
Sample Identification: Pilot Sino Nasal Lot #0017

	TIME INTERVAL						
	CONTROL	0 HOUR	6 HOUR	24 HOUR	72 HOUR	5 DAY	7 DAY
Staphylococcus aureus # 6538							
AVE	1.4×10^6	5.6×10^4	$<2.0 \times 10^2$	$<2.0 \times 10^2$	$<2.0 \times 10^2$	$<2.0 \times 10^2$	$<2.0 \times 10^2$
LOG RED	N/A	1.39	>3.83	>3.83	>3.83	>3.83	>3.83
Pseudomonas aeruginosa							
AVE	2.4×10^5	$<4.7 \times 10^2$	$<2.0 \times 10^2$	$<2.0 \times 10^2$	$<2.0 \times 10^2$	$<2.0 \times 10^1$	$<2.0 \times 10^1$
LOG RED	N/A	>2.71	>3.07	>3.07	>3.07	>4.07	>4.07
Escherichia coli							
AVE	1.1×10^6	1.4×10^3	$<2.0 \times 10^2$	$<2.0 \times 10^2$	$<2.0 \times 10^2$	$<2.0 \times 10^2$	$<2.0 \times 10^2$
LOG RED	N/A	2.90	>3.74	>3.74	>3.74	>3.74	>3.74
Candida albicans							
AVE	1.3×10^6	5.8×10^6	$<2.0 \times 10^2$	2.0×10^2	$<2.0 \times 10^2$	$<2.0 \times 10^1$	$<2.0 \times 10^1$
LOG RED	N/A	0.37	>3.83	>3.83	>3.83	>4.83	>4.83
Streptococcus pyogenes							
AVE	4.3×10^5	$<2.0 \times 10^2$	$<2.0 \times 10^2$	$<2.0 \times 10^2$	$<2.0 \times 10^2$	$<2.0 \times 10^2$	$<2.0 \times 10^2$
LOG RED	N/A	>3.33	>3.33	>3.33	>3.33	>3.33	>3.33
Alternaria alternata							
AVE	3.5×10^2	6.0×10^1	$<2.0 \times 10^1$	$<2.0 \times 10^1$	$<2.0 \times 10^1$	$<2.0 \times 10^1$	$<2.0 \times 10^1$
LOG RED	N/A	0.77	>1.25	>1.25	>1.25	>1.25	>1.25
Cladosporium herbarum							
AVE	$<4.0 \times 10^2$	3.0×10^2	$<2.0 \times 10^2$	$<2.0 \times 10^2$	$<2.0 \times 10^1$	$<2.0 \times 10^1$	$<2.0 \times 10^1$
LOG RED	N/A	0.13	>0.30	>0.30	>1.30	>1.30	>1.30

The averages listed are the total average for both replicates. The values are expressed in CFU/mL.

TABLE 2. Summary of Results (continued)
0 hour, 6 hour, 24 hour, 72 hour, 5 day, and 7 day
Sample Identification: Pilot Sino Nasal Lot #0017

	TIME INTERVAL						
	CONTROL	0 HOUR	6 HOUR	24 HOUR	72 HOUR	5 DAY	7 DAY
Penicillium funiculosum							
AVE	8.2×10^3	$<2.0 \times 10^2$	$<2.0 \times 10^2$	$<2.0 \times 10^2$	$<2.0 \times 10^2$	$<2.0 \times 10^2$	$<2.0 \times 10^2$
LOG RED	N/A	>1.61	>1.61	>1.61	>1.61	>1.61	>1.61
Fusarium solani							
AVE	2.1×10^4	2.0×10^3	$<2.0 \times 10^2$	$<2.0 \times 10^2$	$<2.0 \times 10^2$	$<2.0 \times 10^2$	$<2.0 \times 10^2$
LOG RED	N/A	1.04	>2.03	>2.03	>2.03	>2.03	>2.03
Haemophilus influenzae							
AVE	2.3×10^5	4.3×10^5	$<2.0 \times 10^3$	$<2.0 \times 10^3$	$<2.0 \times 10^3$	$<2.0 \times 10^3$	$<2.0 \times 10^3$
LOG RED	N/A	-0.26	>2.07	>2.07	>2.07	>2.07	>2.07
Moraxella cattarrhalis							
AVE	1.2×10^5	$<2.0 \times 10^2$	$<2.0 \times 10^2$	$<2.0 \times 10^2$	$<2.0 \times 10^2$	$<2.0 \times 10^2$	$<2.0 \times 10^2$
LOG RED	N/A	N/A	>2.79	>2.79	>2.79	>2.79	>2.79
Staphylococcus aureus # 700698							
AVE	4.0×10^5	7.2×10^4	$<2.0 \times 10^2$	$<2.0 \times 10^2$	$<2.0 \times 10^2$	$<2.0 \times 10^1$	$<2.0 \times 10^1$
LOG RED	N/A	0.75	>3.30	>3.30	>3.30	>4.30	>4.30
Aspergillus niger							
AVE	6.1×10^5	3.1×10^5	3.2×10^3	$<2.0 \times 10^2$	$<2.0 \times 10^2$	$<2.0 \times 10^2$	$<2.0 \times 10^2$
LOG RED	N/A	0.29	2.28	>3.49	>3.49	>3.49	>3.49
Stachybotrys chartarum							
AVE	1.1×10^4	$<2.0 \times 10^2$	$<2.0 \times 10^2$	$<2.0 \times 10^2$	$<2.0 \times 10^1$	$<2.0 \times 10^1$	$<2.0 \times 10^1$
LOG RED	N/A	>1.76	>1.76	>1.76	>2.76	>2.76	>2.76

The averages listed are the total average for both replicates. The values are expressed in CFU/mL.

TABLE 3. Neutralization Data
Sample Identification: Pilot Sino Nasal, Lot #0017

ORGANISMS	CONTROL	SAMPLE	PERCENT OF CONTROL
F. solani	1.3	1.3	100.0%
A. alternata	12.7	11.3	89.0%
P. funiculosum	72.7	53.7	73.9%
S. pyogenes	26.7	20.0	74.9%
S. aureus ATCC #700698	23.7	24.7	104.2%
M. cattarrhalis	7.7	7.3	94.8%
S. aureus	55.0	48.3	87.9%
P. aeruginosa	21.3	18.3	85.9%
E. coli	71.7	58.0	80.9%
S. chartarum	384.0	432.0	112.5%
C. herbarum	102.0	77.3	75.8%
A. niger	13.0	11.3	86.9%
C. albicans	79.3	73.0	92.1%
H. influenza	0.33	0.33	100.0%

The values are expressed in CFU/0.5 mL.