



FINAL REPORT

QUANTITATIVE MINI KILL TIME TEST: FUNGAL

PROTOCOL NO. 200220302-01

LABORATORY NO. 215293.1 AMENDED

PREPARED FOR:

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NELSON LABORATORIES, INC.

STUDY DIRECTOR GLP CERTIFICATION

USFDA (21 CFR PART 58)

USEPA (40 CFR PART 160)

QUANTITATIVE MINI KILL TIME TEST: FUNGAL

I CERTIFY THAT THE TEST WAS CONDUCTED IN ACCORDANCE
WITH THE USFDA OR USEPA REGULATIONS AS NOTED ABOVE.

LABORATORY NO. 215293.1 AMENDED

STUDY DIRECTOR:

DATE:

07 Nov 2003



NELSON LABORATORIES, INC.

QAU AUDIT STATEMENT

USFDA (21 CFR PART 58)

USEPA (40 CFR PART 160)

QUANTITATIVE MINI KILL TIME TEST: FUNGAL

Study Director:

Final Report Dated:

Tonya M. Rixey, B.S. RM(NRM)

20 Sep 2002

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 215293.1 Amended.
2. In accordance with the Good Laboratory Practice Regulations, the Plating phase(s) of this study was inspected by the Quality Assurance Unit on: 23 Aug 2002. The findings of the inspection(s) were reported to Management and to the Study Director on: 23 Aug 2002.
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: 04/20/2003



QUANTITATIVE MINI KILL TIME TEST: FUNGAL

LABORATORY NUMBER:	215293.1 Amended
PROTOCOL NUMBER:	200220302-01
SAMPLE SOURCE:	SinoFresh Laboratories, Inc.
SAMPLE IDENTIFICATION:	Pilot Sino Nasal lot #0017
DEVIATIONS:	None
DATA ARCHIVE LOCATION:	Sequentially by lab number
PROTOCOL APPROVAL DATE:	06 Aug 2002
SAMPLE RECEIVED DATE:	01 Aug 2002
LAB PHASE START DATE:	06 Aug 2002
LAB PHASE COMPLETION DATE:	19 Sep 2002
REPORT ISSUE DATE:	20 Sep 2002
STUDY COMPLETION DATE:	20 Sep 2002
AMENDED REPORT ISSUE DATE:	03 Nov 2003
TOTAL NUMBER OF PAGES:	9

AMENDMENT JUSTIFICATION:

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

REFERENCES:

United States Pharmacopeia 25 & National Formulary 20. 2002. 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 pilot Sino Nasal product. The test employed methods described in the United States Pharmacopoeia.

Samples of the product were inoculated with 6 fungal test organisms. Aliquots, of the inoculated product, were removed at 0 hours, 6 hours, 24 hours, 72 hours, 5 days, and 7 days and assayed for surviving organisms. The log reduction in the level of the test organisms was calculated for each time interval.

PROCEDURE:

The following organisms were tested:

- 1) *Alternaria alternata* ATCC #44501
(Mold)
- 2) *Cladosporium herbarum* ATCC #28987
(Mold)
- 3) *Penicillium funiculosum* ATCC #10509
(Mold)
- 4) *Candida albicans* ATCC #10231
(Yeast)
- 5) *Aspergillus niger* ATCC #16404
(Mold)
- 6) *Fusarium solani* ATCC #36031
(Mold)

The *C. herbarum* was transferred to Malt Extract agar. The *C. albicans* and other molds were transferred to Sabouraud dextrose agar (SDEX). The *C. albicans* was incubated at 20-25°C for 48 hours. The molds were incubated at 20-25°C for 7 days.

The *C. albicans* was harvested using 0.9% saline. The molds were harvested with 0.9% saline containing 0.05% Tween® 80. The cultures were filtered through gauze and vortexed vigorously to break up clumps. The titer of each suspension was adjusted to approximately 10⁸ colony forming units (CFU)/mL using visual turbidity. The actual titer of each culture was determined using the positive control values.

Two 10 mL samples of the test product were prepared for each of the challenge organisms. The duplicate tubes containing 10 mL samples were inoculated with 100 µL of the test organism using a calibrated micropipettor. The volume of the inoculum was 0.5-1.0% of the volume of the product. The final concentration of the controls were between 10³ and 10⁵ CFU/mL. The samples were well mixed. Due to the difficulty in growing and harvesting some of these organisms, some of the titers were approximating 10³. These organisms show a lower log reduction due to the low titer, not due to a lack of kill. All of the organisms showed no growth within 72 hours.

Two positive control tubes were prepared for each organism using sterile water. Negative controls were also prepared. A 100 µL aliquot of the test organism was added to 10 mL of sterile water for the positive control. All test samples were stored at 20-25°C.

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

Sample aliquots at each interval were diluted in letheen broth (LETH) and plated on SDEX. The *C. herbarium* was plated on malt extract agar. 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 uninoculated product to 9.0 mL of LETH blanks. This simulates the highest concentrations of the product tested, and represents the worst case for neutralization. The LETH blanks were inoculated with 1.0 mL of an approximate 100-1000 CFU/mL organism suspension, and plated in 0.5 mL aliquots on NUAG. 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 of colonies on the control plates.

RESULTS:

The results for the product are reported in Table 1. All values are shown in CFU/mL. The less than value represents the detectable limit of the test where 0 CFU were observed on the plates.


The neutralization data is found in Table 2. 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 samples. Effective neutralization was achieved in the first dilution (1/10). The detectable limits of the assay are approximately 20 CFU/mL.



Quality Assurance Reviewer



Tonya M. Rixey, B.S. RM(NRM)
Study Director



Amended Report Date

TABLE 1. Summary of Results

	TIME INTERVAL						
	CONTROL	0 HOUR	6 HOUR	24 HOUR	72 HOUR	5 DAY	7 DAY
<i>Alternaria alternata</i>							
Ave	5.3 x 10 ³	<2.0 x 10 ²	<2.0 x 10 ²	<2.0 x 10 ²	<20	<20	<20
Log Red	N/A	>1.4	>1.4	>1.4	>2.4	>2.4	>2.4
<i>Cladosporium herbarium</i>							
Ave	2.1 x 10 ³	1.5 x 10 ³	<2.0 x 10 ²	<2.0 x 10 ²	<20	<20	<20
Log Red	N/A	0.1	>1.0	>1.0	>2.0	>2.0	>2.0
<i>Penicillium funiculosum</i>							
Ave	3.9 x 10 ⁵	3.7 x 10 ³	<2.0 x 10 ²	<2.0 x 10 ²	<20	<20	<20
Log Red	N/A	2.0	>3.3	>3.3	>4.3	>4.3	>4.3
<i>Candida albicans</i>							
Ave	1.2 x 10 ⁶	2.7 x 10 ³	<2.0 x 10 ²	<2.0 x 10 ²	<20	<20	<20
Log Red	N/A	2.6	>3.8	>3.8	>4.8	>4.8	>4.8
<i>Fusarium solani</i>							
Ave	4.0 x 10 ⁴	<9.7 x 10 ²	<2.0 x 10 ²	<2.0 x 10 ²	<20	<20	<20
Log Red	N/A	>1.6	>2.3	>2.3	>3.3	>3.3	>3.3
<i>Aspergillus niger</i>							
Ave	6.1 x 10 ⁴	1.3 x 10 ⁵	2.4 x 10 ⁴	<2.0 x 10 ²	<20	<20	<20
Log Red	N/A	-0.3	0.4	>2.5	>3.5	>3.5	>3.5

TABLE 2. Neutralization Data
Pilot Sino Nasal Lot #0017

ORGANISMS	CONTROL	SAMPLE	PERCENT OF CONTROL
<i>A. alternata</i>	21	16	77%
<i>C. herbarium</i>	11	8	73%
<i>P. funiculosum</i>	9	7	78%
<i>C. albicans</i>	43	42	98%
<i>F. solani</i>	16	11	67%
<i>A. niger</i>	20	17	84%

Note: The values are expressed in CFU/0.5 mL.

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