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FINAL REPORT

STUDY TITLE

FUNGICIDAL ACTIVITY OF A DISINFECTANT

AUTHOR

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STUDY COMPLETED ON

11 JAN 2002

PERFORMING LABORATORY

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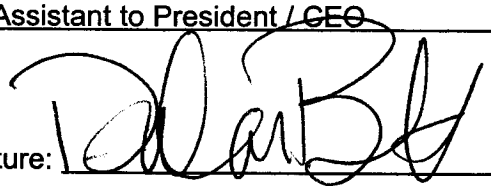
Company: Innovative Medical Services

Company Agent: Dolana Blount

Title: Assistant to President / ~~CEO~~

Signature: 

Date: 01-23-2002

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I CERTIFY THAT THIS STUDY WAS PERFORMED IN ACCORDANCE

WITH THE U.S. EPA GOOD LABORATORY PRACTICES.

(GLP REGULATIONS)

LABORATORY NO. 197157

Shelli Baxter, B.S. SM(NRM)
Nelson Laboratories, Inc.

Shelli Baxter
Signature

Study Director
Title

14 Jan 2002
Date

Dolana Blount
Submitter's Name

Signature

Assistant to President / CEO
Title

01-23-2002
Date

Dolana Blount
Sponsor's Name

Signature

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01-23-2002.
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PLEASE SIGN AND DATE BOTH COPIES OF THIS DOCUMENT. RETURN ONE ORIGINAL TO THE CUSTOMER SERVICE DEPARTMENT AT NELSON LABS.

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STUDY DIRECTOR GLP CERTIFICATION

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

FUNGICIDAL ACTIVITY OF A DISINFECTANT

I CERTIFY THAT THE TEST WAS CONDUCTED IN ACCORDANCE

WITH THE USFDA OR USEPA REGULATIONS AS NOTED ABOVE.

LABORATORY NO. 197157

STUDY DIRECTOR: Shelli Baxter DATE: 14 Jan 2002

SOP/QAU/018G.2-9/102000

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QAU AUDIT STATEMENT

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

FUNGICIDAL ACTIVITY OF A DISINFECTANT

Study Director: Shelli Baxter, B.S. SM(NRM)

Final Report Dated: 11 Jan 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 197157.

2. In accordance with the Good Laboratory Practice Regulations, this study was inspected by the Quality Assurance Unit on: 07 Jan 2002. The findings of the inspection(s) were reported to Management and to the Study Director on: 07 Jan 2002 and 10 Jan 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: M. Kay Mankel DATE: 15 Jan 2002

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FUNGICIDAL ACTIVITY OF A DISINFECTANT

LABORATORY NUMBER:	197157
PROTOCOL NUMBER:	200124703-03
SAMPLE SOURCE:	Innovative Medical Services
SAMPLE IDENTIFICATION:	Lot #1: 2001-042-001; Lot #2: 2001-005-001
DEVIATIONS:	None
DATA ARCHIVE LOCATION:	Sequentially by lab number
NUMBER OF TEST SAMPLES:	2
PROTOCOL APPROVAL DATE:	20 Nov 2001
SAMPLE RECEIVED DATE:	19 Nov 2001
LAB PHASE START DATE:	28 Nov 2001
LAB PHASE COMPLETION DATE:	11 Jan 2002
REPORT ISSUE DATE:	11 Jan 2002
TOTAL NUMBER OF PAGES:	13

REFERENCES:

AOAC International. 2000. Official Methods of Analysis. Volume 1, Chapter 6, 955.17 Fungicidal Activity of Disinfectants. AOAC International, Gaithersburg, MD.

United States Environmental Protection Agency. Office of Pesticide Programs. DIS/TSS-6 Efficacy Data Requirements. Supplemental Efficacy (A) Pathogenic Fungi.

United States Environmental Protection Agency. Office of Pesticide Programs. Draft Subpart W - CFR 158 Antimicrobials Data Requirements.

INTRODUCTION:

This report details the evaluation of procedures for the evaluation of AXEN® EPA Registration Number 72977-2 from Innovative Medical Services for fungicidal efficacy. Two lots of product were tested at 30 ppm against *Trichophyton mentagrophytes* ATCC #9533, following the procedures outlined in the AOAC Official Methods of Analysis, Fungicidal Activity of Disinfectants (955.17).

PROCEDURES:

INOCULUM PREPARATION:

Trichophyton mentagrophytes ATCC #9533 was transferred to 5 plates of glucose agar and incubated at 27-29°C for 14 days. After incubation, the mycelial mats were removed from the agar

surface using a sterile spatula, transferred to a sterile tissue grinder and macerated using 25 mL of PHSS. The suspension was filtered through a sterile funnel containing moist cotton and the suspension was standardized with PHSS to contain $\approx 10^6$ conidia/mL. A standard plate count was performed on the conidial suspension to verify the titer of the test organism.

SAMPLE PREPARATION:

On the day of test, a 30 ppm solution of AXEN® was prepared by diluting the 2410 ppm concentrate with 5% (w/w) citric acid in purified water. A 30 ppm solution was prepared from both lots of concentrate (2001-042-001 and 2001-005-001).

TEST PERFORMANCE:

Five mL of each of the test solutions were placed in sixty 25 x 150 mm test tubes and the tubes were placed in a 20 ± 1°C waterbath. Using a calibrated micropipettor, 0.5 mL of conidial suspension were placed in the first tube of test solution, shaken, and immediately replaced in the waterbath. At 30 second intervals, 0.5 mL of the conidial suspension were added to the second tube. This was repeated at 30 second intervals until all tubes were inoculated. After 30 seconds, 1, 2, 5, and 10 minute intervals, a sample from each tube was removed with a 4 mm loop and placed into 20 ml of glucose broth. The tubes were incubated at 27-29°C for 10 days and then scored as (+) or (0) for growth of the challenge organism.

PHENOL RESISTANCE:

The phenol resistance of the test culture was determined according to the phenol dilutions of 1:60 and 1:70. A 5% stock solution of phenol (1:20) was diluted further to make the needed dilutions. Five milliliter aliquots of each dilution were placed into sterile test tubes and allowed to equilibrate in a 20 ± 0.5°C waterbath. An additional tube was prepared and the thermometer was placed in that tube to show when the phenol dilutions had equilibrated to the test temperature. One half milliliter of the conidial suspension was added to each tube at 30 second intervals. The tubes were gently agitated to distribute the culture and replaced in the waterbath. The exposure times were 5, 10, and 15 minutes. After the appropriate exposure time, a loopful (4 mm loop bent at a 30° angle) was removed from the assay tube and transferred to a tube of LETH. The tubes of LETH were incubated at 27-29°C for 4 days. Growth was reported as being either (+) positive or (0) negative for the challenge organism.

NEUTRALIZATION VERIFICATION:

The neutralization efficacy of the subculture tubes was demonstrated by adding 1-100 CFU of the test organism in each tube. The inoculating titer was verified in triplicate on glucose agar. The tubes and plates were incubated for 3-4 days at 27-29°C and observed for growth.

GROWTH PROMOTION OF MEDIA:

The growth promotion properties of the glucose broth were demonstrated in the media control tubes by adding 1-100 CFU of the test organism in each tube. The inoculating titer was verified in triplicate on glucose agar. The tubes and plates were incubated for 4 days at 27-29°C and observed for growth.

STERILITY CONTROLS:

Two tubes of glucose broth were included with the test as a media control. All tubes were incubated with the test in order to confirm sterility of the recovery media used in the test.

RESULTS:

Test results can be found in Table 1. AXEN® 30 ppm solution lot #2001-042-001 demonstrated kill of *T. mentagrophytes* in 1 of 60 tubes after a 30 second exposure, 2 of 60 tubes after a 1 minute exposure, 6 of 60 tubes after a 2 minute exposure, 49 of 60 tubes after a 5 minute exposure, and 60 of 60 tubes after a 10 minute exposure. AXEN® 30 ppm solution lot #2001-005-001 demonstrated kill of *T. mentagrophytes* in 0 of 60 tubes after a 30 second exposure, 0 of 60 tubes after a 1 minute exposure, 3 of 60 tubes after a 2 minute exposure, 50 of 60 tubes after a 5 minute exposure, and 60 of 60 tubes after a 10 minute exposure. The titer of *T. mentagropytes* in each tube of test solution was 1.2×10^7 conidia/tube.

Phenol resistance results can be found in Table 2. The condia must survive a 10 minute exposure to phenol dilution 1:70, but not 1:60. The test culture survived a 10 minute exposure to both phenol dilutions, providing a more severe challenge for the test.

Neutralization and growth promotion results can be found in Table 3. All media tubes demonstrated growth by 4 days. The inoculating titer of *T. mentagropytes* was 10-100 CFU/tube.

Deborah Petric
Technical Reviewer

Shelli Baxter, B.S. SM(NRM)
Study Director

14 Jan 2002
Study Completion Date

SAB/mjm

TABLE 1. Fungicidal Efficacy Results

AXEN® LOT NUMBER	TIME INTERVAL	NUMBER OF TUBES TESTED	NUMBER OF TUBES EXHIBITING GROWTH	NUMBER OF TUBES EXHIBITING NO GROWTH
2001-042-001	30 seconds	60	59	1
	1 minute	60	58	2
	2 minutes	60	54	6
	5 minutes	60	11	49
	10 minutes	60	0	60
2001-005-001	30 seconds	60	60	0
	1 minute	60	60	0
	2 minutes	60	57	3
	5 minutes	60	10	50
	10 minutes	60	0	60
Positive Control	N/A	3	3	0
Negative Control		3	0	3

TABLE 2. Phenol Resistance Results

TIME IN MINUTES	PHENOL DILUTION	
	1:60	1:70
5	+	+
10	+	+
15	+	+

TABLE 3. Neutralization and Growth Promotion Results

TUBE	EXPOSURE TIME	CFU/TUBE	RESULTS
AXEN® Lot 2001-042-001	30 seconds	78	1/1 (+)
	1 minute	78	2/2 (+)
	2 minutes	78	6/6 (+)
	5 minutes	78	49/49 (+)
	10 minutes	47	60/60 (+)
AXEN® Lot 2001-005-001	30 seconds	N/A*	N/A*
	1 minute	N/A*	N/A*
	2 minutes	59	3/3 (+)
	5 minutes	59	50/50 (+)
	10 minutes	47	60/60 (+)
Media Control	N/A	78	3/3 (+)

* No neutralization was performed because all the tubes grew in test.