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FINAL REPORT

STUDY TITLE

KILL TIME STUDY FOR *PROPIONIBACTERIUM ACNES*

AUTHOR

SHELLI A. BAXTER, B.S. SM(NRM)
STUDY DIRECTOR

STUDY COMPLETED ON

05 FEB 2002

PERFORMING LABORATORY

NELSON LABORATORIES, INC.
6280 SOUTH REDWOOD ROAD
SALT LAKE CITY, UT 84123-6600
801-963-2600

LABORATORY SAMPLE ID

197156

SUBMITTED TO:

INNOVATIVE MEDICAL SERVICES
1725 GILLESPIE WAY
EL CAJON, CA 92020
619-596-8600 x 105

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STATEMENT OF DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA§10(d)(1)(A),(B), or (C).

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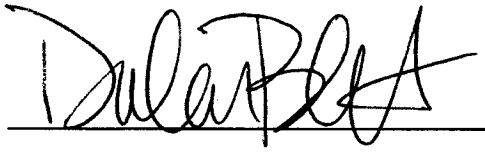
Company: Innovative Medical Services

Company Agent: Dolana Blount

Title: Assistant to the President

Signature:  Date: 02.08.02

SOP/QAU/018G.2-12/102000

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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. 197156

Shelli A. Baxter, B.S. SM(NRM) Nelson Laboratories, Inc.	Study Director Title
Shelli Baxter Signature	05 Feb 2002 Date
Dolana Blount Submitter's Name	Assistant to the President Title
Dolana Blount Signature	02-08-02 Date
Dolana Blount Sponsor's Name	Assistant to the President Title
Dolana Blount Signature	02-08-02 Date

SOP/QAU/018G.2-13/102000

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

Nelson Laboratories Incorporated
P. O. Box 17557, Salt Lake City, UT 84117-0557 • 6280 South Redwood Road, Salt Lake City, UT 84123-6600
Telephone: 801-963-2600 • Fax: 801-963-2630 • Operator: 800-826-2088 • Auto Attendant: 800-963-6280 or 801-963-5299
Web site: www.nelsonlabs.com • e-mail: info@nelsonlabs.com

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STUDY DIRECTOR GLP CERTIFICATION

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

KILL TIME STUDY FOR *PROPIONIBACTERIUM ACNES*

I CERTIFY THAT THE TEST WAS CONDUCTED IN ACCORDANCE

WITH THE USFDA OR USEPA REGULATIONS AS NOTED ABOVE.

LABORATORY NO. 197156

STUDY DIRECTOR: Shelli Baxter DATE: 05 Feb 2002

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Nelson Laboratories Incorporated
P. O. Box 17557, Salt Lake City, UT 84117-0557 • 6280 South Redwood Road, Salt Lake City, UT 84123-6600
Telephone: 801-963-2600 • Fax: 801-963-2630 • Operator: 800-826-2088 • Auto Attendant: 800-963-6280 or 801-963-5299
Web site: www.nelsonlabs.com • e-mail: info@nelsonlabs.com

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QAU AUDIT STATEMENT

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

KILL TIME STUDY FOR *PROPIONIBACTERIUM ACNES*

Study Director: Final Report Dated:

Shelli A. Baxter, B.S. SM(NRM) 05 Feb 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 197156.

2. In accordance with the Good Laboratory Practice Regulations, this study was inspected by the Quality Assurance Unit on: 11 Jan 2002. The findings of the inspection(s) were reported to Management and to the Study Director on: 11 Jan 2002 and 12 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. Faye Hackel DATE: 06 Feb 2002

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KILL TIME STUDY FOR *PROPIONIBACTERIUM ACNES*

LABORATORY NUMBER:	197156
PROTOCOL NUMBER:	200135201-01
SAMPLE SOURCE:	Innovative Medical Services
SAMPLE IDENTIFICATION:	Lot #2001-042-001 Lot #2001-005-001
DEVIATIONS:	None
DATA ARCHIVE LOCATION:	Sequentially by lab number
NUMBER OF TEST SAMPLES:	2
PROTOCOL APPROVAL DATE:	18 Dec 2001
SAMPLE RECEIVED DATE:	19 Nov 2001
LAB PHASE START DATE:	18 Dec 2001
LAB PHASE COMPLETION DATE:	30 Jan 2002
REPORT ISSUE DATE:	05 Feb 2002
TOTAL NUMBER OF PAGES:	10

REFERENCE:

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

INTRODUCTION:

This report describes the procedures for the evaluation of AXEN® EPA Registration Number 72977-2 from Innovative Medical Services for efficacy against *Propionibacterium acnes*. Two lots of product were tested at 30 ppm against *P. acnes* ATCC #6921 employing a liquid to liquid matrix. The product was exposed to the test organism in duplicate at six exposure times and quantitated using standard plate count procedures.

PROCEDURES:

INOCULUM PREPARATION:

P. acnes ATCC #6921 was transferred to modified thioglycollate broth and incubated anaerobically at 35-39°C for 9 days to yield a high titer. The organism was vortexed thoroughly and then diluted 1:10 in physiological saline (PHSS) to yield a starting titer of 10^7 CFU/mL. Ten-fold serial dilutions of the adjusted suspension were prepared in dilution blanks containing 9 mL of PHSS. Triplicate aliquots from selected dilutions were plated onto trypticase soy agar with 5% defibrinated sheep blood. The plates were incubated anaerobically at 35-39°C for 3 days and the starting titer of the test organism was calculated.

POSITIVE CONTROL:

One tube was prepared containing 9 mL of PHSS. The tube was equilibrated to 20 ± 1°C. At T=0, 1 mL of test organism was added to the tube. The sample was held for the longest time interval specified by the sponsor (90 seconds). Ten-fold serial dilutions were prepared in dilution blanks containing 9 mL of neutralizer broth (NEUB). Triplicate aliquots from selected dilutions were plated onto trypticase soy agar with 5% defibrinated sheep blood and the plates were incubated anaerobically at 35-39°C for 3 days.

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 PROCEDURE:

One 25 x 150 mm tube containing 9.0 mL of each test sample was brought to 20 ± 1°C in a waterbath. One mL of test organism was added to each tube containing the test sample to yield a minimum of 1 x 10^6 CFU/mL challenge of *P. acnes.* The samples were mixed by swirling. The tubes were placed back into the waterbath. At 15, 30, 45, 60, 75, and 90 seconds of exposure, 1.0 mL aliquots of disinfectant-cell suspension were removed and added to 9 mL of NEUB. The tubes were mixed thoroughly. Triplicate aliquots were plated onto trypticase soy agar with 5%

defibrinated sheep blood. Additionally, the remaining volume was filtered through a 0.45 μm membrane filter and the filter was placed onto a blood agar plate. The plates were incubated anaerobically at 35-39°C for 3 days.

NEUTRALIZATION VERIFICATION:

One mL of test organism was added to 9 mL of NEUB to achieve a titer of 1-100 CFU/mL. One mL of the disinfectant was placed into 8 mL of NEUB. The disinfectant/neutralizer broth was allowed to equilibrate to the same temperature as the test samples. One mL of test organism was added to the mixture to achieve a titer of 1-100 CFU/mL. Incubation was continued for the approximate time it would take to plate a sample. Triplicate aliquots from selected dilutions were plated onto trypticase soy agar with 5% defibrinated sheep blood and the plates were incubated anaerobically at 35-39°C for 3 days. The primary recovery tube was tested to demonstrate neutralization at the highest concentration of disinfectant.

RESULTS:

The percent reduction and log reduction results for each lot of AXEN®can be found in Table 1. The organism titer was determined to be 2.0×10^7 CFU/mL. The positive control titer was determined to be 1.9×10^6 CFU/mL. The percent neutralization recovery was 72% for Lot #2001-042-001 and 94% for Lot #2001-005-001.

Deborah Petric
Technical Reviewer

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

05 Feb 2002
Study Completion Date

SAB/clc

Table 1. *P. acnes* Time Kill
AXEN® 30 ppm prepared with 5% (w/w) Citric Acid in Purified Water

SAMPLE IDENTIFICATION	INITIAL COUNTS (CFU/mL)	CONTACT TIME (Seconds)	FINAL COUNTS (CFU/mL)	PERCENT (%) REDUCTION	LOG_{10} REDUCTION
AXEN® Lot #2001-005-001	1.9×10^6	15	3	99.9998%	5.80
	1.9×10^6	30	<1	>99.99995%	6.28
	1.9×10^6	45	<1	>99.99995%	6.28
	1.9×10^6	60	<1	>99.99995%	6.28
	1.9×10^6	75	<1	>99.99995%	6.28
	1.9×10^6	90	<1	>99.99995%	6.28
AXEN® Lot #2001-042-001	1.9×10^6	15	1	99.99995%	6.28
	1.9×10^6	30	<1	>99.99995%	6.28
	1.9×10^6	45	<1	>99.99995%	6.28
	1.9×10^6	60	<1	>99.99995%	6.28
	1.9×10^6	75	<1	>99.99995%	6.28
	1.9×10^6	90	<1	>99.99995%	6.28