



FINAL REPORT

STUDY TITLE

EVALUATION OF AXEN® FOR RESIDUAL ACTIVITY

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STUDY COMPLETED ON

08 FEB 2002

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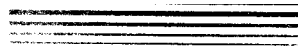
Company: Innovative Medical Services

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Date: 03/04/02
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(GLP REGULATIONS)

LABORATORY NO. 197158

Shelli Baxter, B.S. Sm(NRM)
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08 Feb 2002
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USFDA (21 CFR PART 58)

USEPA (40 CFR PART 160)

EVALUATION OF AXEN[®] FOR RESIDUAL ACTIVITY

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

LABORATORY NO. 197158

STUDY DIRECTOR:

Shelli Barton

DATE:

08 Feb 2002

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QAU AUDIT STATEMENT

[] USFDA (21 CFR PART 58)

[X] USEPA (40 CFR PART 160)

EVALUATION OF AXEN® FOR RESIDUAL ACTIVITY

Study Director:

Final Report Dated:

Shelli Baxter, B.S. RM(NRM)

08 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 197158.
2. In accordance with the Good Laboratory Practice Regulations, this study was inspected by the Quality Assurance Unit on: 21 Dec 2001. The findings of the inspection(s) were reported to Management and to the Study Director on: 21 Dec 2001 and 27 Dec 2001.
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. Kaye Markel* DATE: *21 Feb 2002*
SOP/QAU/018G.2-10/102000

EVALUATION OF AXEN® FOR RESIDUAL ACTIVITY

LABORATORY NUMBER: 197158
PROTOCOL NUMBER: 200132009-02
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: 30 Nov 2001
SAMPLE RECEIVED DATE: 19 Nov 2001
LAB PHASE START DATE: 10 Dec 2001
LAB PHASE COMPLETION DATE: 08 Feb 2002
REPORT ISSUE DATE: 08 Feb 2002
TOTAL NUMBER OF PAGES: 22

REFERENCES:

AOAC Official Methods of Analysis. 1995. AOAC Official Method 961.02 Germicidal Spray Products. Chapter 6. p. 11-12. AOAC International.

INTRODUCTION:

This report details the evaluation of AXEN® EPA Registration Number 72977-2 from Innovative Medical Services for residual activity after initial application of the disinfectant to a hard surface. Two lots of product were tested at 30 ppm against the following three organisms:

Staphylococcus aureus ATCC #6538
Salmonella choleraesuis ATCC #10708
Pseudomonas aeruginosa ATCC #15442

AXEN® was applied to glass slides and wiped with a clean towel. The test organisms were applied to 54 slides (9 per organism per lot of AXEN®) at 0, 1, 6, and 24 hours. Eighteen (3 per organism per lot of AXEN®) slides were held for 30 seconds, 1 minute, and 2 minutes after inoculation, then analyzed for kill of the organism.

PROCEDURES:

CULTURE PREPARATION:

From stock cultures, tubes (one per organism) of NBOAC were inoculated with *Staphylococcus aureus* ATCC #6538, *Salmonella choleraesuis* ATCC #10708, and *Pseudomonas aeruginosa* ATCC #15442. The tubes were incubated at $37 \pm 2^{\circ}\text{C}$ for approximately 48 hours. The *Pseudomonas* test culture was aseptically decanted, leaving the pellicle behind. All test cultures were vortexed 3-4 seconds and allowed to stand 10 minutes at room temperature.

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 SLIDE PREPARATION:

The glass slides were sterilized in a standard Nelson Laboratories dry load. The sterilized slides were transferred to individual sterile petri dishes matted with 2 pieces of sterile Whatman filter paper. Eighteen slides per organism, per lot of AXEN®, per time point were prepared, for a total of 216 slides. Each petri dish was labeled with the organism, lot number of disinfectant, and time point.

TEST PERFORMANCE:

One milliliter of the appropriate lot of AXEN® was applied to each slide. The disinfectant was spread evenly over the entire surface of the slide with a clean towel. At 0, 1, 6, and 24 hours, 0.01 mL of test culture were transferred onto the sterile test slides in the petri dishes and immediately spread uniformly over an approximate 1" x 1" area. The dishes were covered and the inoculation repeated until all 18 slides were prepared for each organism. Three slides per organism, per lot of AXEN® were held for 30 seconds, 1 minute, and 2 minutes. After the exposure interval, the slides were individually transferred to a bottle of lethene (LETH) with sterile forceps. One slide per organism, per time point was extracted as a quantitative control by shaking manually for 1 minute.

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A plate count was performed in triplicate using an appropriate aliquot evenly spread on SCDA plates with a sterile bent glass rod. Aliquots of the remaining extract were filtered through a 0.45 μm filter, the filter was rinsed with 100 mL of peptone Tween® solution, and the filter was placed on a SCDA plate.

The remaining bottles and all plates were incubated for 48-54 hours at $37 \pm 2^\circ\text{C}$ and observed for growth. Three bottles of LETH were incubated with the test as negative controls.

NEUTRALIZATION VERIFICATION:

The neutralization efficacy of the negative subculture bottles was demonstrated by adding 1-100 CFU of the appropriate test organism to each bottle. The inoculating titer was verified in triplicate on SCDA. The bottles and plates were incubated for three days at $37 \pm 2^\circ\text{C}$ and observed for growth.


GROWTH PROMOTION OF MEDIA:

The growth promotion properties of LETH were demonstrated in the media control bottles by adding 1-100 CFU of the appropriate test organism. The inoculating titer was verified in triplicate on SCDA. The bottles and plates were incubated for 3 days at $37 \pm 2^\circ\text{C}$ and observed for growth.

RESULTS:

Test results can be found in Table 1-8. Neutralization and growth promotion results can be found in Table 9. All media bottles demonstrated growth by 3 days.


Deborah Petric
Technical Reviewer


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

08 Feb 2002
Study Completion Date

SB/ejb

TABLE 1. Qualitative Residual Results at 0 Hours
Following Application of AXEN® 30 ppm

Test Organism	Exposure Time on Slide	Growth Results for Lot 2001-042-001	Growth Results for Lot 2001-005-001
<i>S. aureus</i>	30 seconds	Growth	Growth
		Growth	Growth
		Extracted (Table 2)	No Growth
	1 minute	Growth	Growth
		Growth	Growth
		Extracted (Table 2)	No Growth
	2 minutes	Growth	No Growth
		No Growth	No Growth
		Extracted (Table 2)	No Growth
<i>S. choleraesuis</i>	30 seconds	Growth	Growth
		Growth	Growth
		Extracted (Table 2)	Growth
	1 minute	Growth	Growth
		Growth	Growth
		Extracted (Table 2)	Growth
	2 minutes	Growth	Growth
		Growth	Growth
		Extracted (Table 2)	Growth

TABLE 1. Qualitative Residual Results at 0 Hours
Following Application of AXEN® 30 ppm (Cont.)

<i>P. aeruginosa</i>	30 seconds	Growth	Growth
		Growth	Growth
		Extracted (Table 2)	Growth
	1 minute	Growth	No Growth
		Growth	Growth
		Extracted (Table 2)	Growth
	2 minutes	No Growth	No Growth
		Growth	No Growth
		Extracted (Table 2)	No Growth

TABLE 2. Quantitative Residual Results at 0 Hours
Following Application of AXEN® 30 ppm

Test Organism	Initial Counts (CFU/mL)	Contact Time (minutes)	Final Counts (CFU/mL)	Percent (%) Reduction	Log ₁₀ Reduction
<i>S. aureus</i>	7.7 x 10 ³	0.5	2.2 x 10 ¹	99.7	2.54
	7.7 x 10 ³	1	1	99.99	3.89
	7.7 x 10 ³	2	1	99.99	3.89
<i>S. choleraesuis</i>	1.1 x 10 ⁵	0.5	4.0 x 10 ⁴	64	0.44
	1.1 x 10 ⁵	1	5.6 x 10 ²	99.5	2.29
	1.1 x 10 ⁵	2	8	99.99	4.14
<i>P. aeruginosa</i>	5.7 x 10 ⁴	0.5	7.0 x 10 ²	99	1.91
	5.7 x 10 ⁴	1	9.2 x 10 ¹	99.8	2.80
	5.7 x 10 ⁴	2	4	99.99	4.15

**TABLE 3. Qualitative Residual Results at 1 Hour
Following Application of AXEN® 30 ppm**

Test Organism	Exposure Time on Slide	Growth Results for Lot 2001-042-001	Growth Results for Lot 2001-005-001
<i>S. aureus</i>	30 seconds	Growth	Growth
		Growth	Growth
		Extracted (Table 2)	Growth
	1 minute	No Growth	No Growth
		No Growth	Growth
		Extracted (Table 2)	Growth
	2 minutes	No Growth	No Growth
		Growth	No Growth
		Extracted (Table 2)	Growth
<i>S. choleraesuis</i>	30 seconds	Growth	Growth
		Growth	Growth
		Extracted (Table 2)	Growth
	1 minute	Growth	Growth
		Growth	No Growth
		Extracted (Table 2)	Growth
	2 minutes	No Growth	No Growth
		Growth	Growth
		Extracted (Table 2)	Growth

TABLE 3. Qualitative Residual Results at 1 Hour
Following Application of AXEN® 30 ppm (Cont.)

<i>P. aeruginosa</i>	30 seconds	No Growth	Growth
		Growth	Growth
		Extracted (Table 2)	Growth
	1 minute	Growth	Growth
		Growth	No Growth
		Extracted (Table 2)	No Growth
	2 minutes	No Growth	No Growth
		No Growth	No Growth
		Extracted (Table 2)	No Growth

TABLE 4. Quantitative Residual Results at 1 Hour
 Following Application of AXEN[®] 30 ppm (Cont.)

TEST ORGANISM	INITIAL COUNTS (CFU/mL)	CONTACT TIME (minutes)	FINAL COUNTS (CFU/mL)	PERCENT (%) REDUCTION	Log ₁₀ Reduction
<i>S. aureus</i>	7.7 x 10 ³	0.5	1.5 x 10 ¹	99.8	2.71
	7.7 x 10 ³	1	5	99.94	3.19
	7.7 x 10 ³	2	2	99.97	3.59
<i>S. choleraesuis</i>	1.1 x 10 ⁵	0.5	6.3 x 10 ²	99.4	2.24
	1.1 x 10 ⁵	1	3	99.997	4.56
	1.1 x 10 ⁵	2	4	99.996	4.44
<i>P. aeruginosa</i>	5.7 x 10 ⁴	0.5	1.8 x 10 ²	99.7	2.50
	5.7 x 10 ⁴	1	6.7 x 10 ²	99	1.93
	5.7 x 10 ⁴	2	6.2 x 10 ¹	99.9	2.96

TABLE 5. Qualitative Residual Results at 6 Hours
Following Application of AXEN® 30 ppm

TEST ORGANISM	EXPOSURE TIME ON SLIDE	GROWTH RESULTS FOR LOT 2001-042-001	GROWTH RESULTS FOR LOT 2001-005-001
<i>S. aureus</i>	30 seconds	Growth	No Growth
		No Growth	No Growth
		Extracted (Table 2)	No Growth
	1 minute	No Growth	Growth
		No Growth	Growth
		Extracted (Table 2)	No Growth
	2 minutes	Growth	Growth
		Growth	Growth
		Extracted (Table 2)	Growth
<i>S. choleraesuis</i>	30 seconds	Growth	Growth
		Growth	Growth
		Extracted (Table 2)	Growth
	1 minute	No Growth	Growth
		Growth	Growth
		Extracted (Table 2)	Growth
	2 minutes	Growth	Growth
		No Growth	No Growth
		Extracted (Table 2)	Growth

TABLE 5. Qualitative Residual Results at 6 Hours
Following Application of AXEN® 30 ppm (Cont.)

TEST ORGANISM	EXPOSURE TIME ON SLIDE	GROWTH RESULTS FOR LOT 2001-042-001	GROWTH RESULTS FOR LOT 2001-005-001
<i>P. aeruginosa</i>	30 seconds	Growth	No Growth
		Growth	Growth
		Extracted (Table 2)	Growth
	1 minute	Growth	No Growth
		Growth	Growth
		Extracted (Table 2)	No Growth
	2 minutes	No Growth	No Growth
		No Growth	No Growth
		Extracted (Table 2)	No Growth

TABLE 6. Quantitative Residual Results at 6 Hours
Following Application of AXEN® 30 ppm

TEST ORGANISM	INITIAL COUNTS (CFU/mL)	CONTACT TIME (minutes)	FINAL COUNTS (CFU/mL)	PERCENT (%) REDUCTION	LOG ₁₀ REDUCTION
<i>S. aureus</i>	3.2×10^4	0.5	2	99.993	4.20
	3.2×10^4	1	1	99.997	4.50
	3.2×10^4	2	1.3×10^1	99.96	3.39
<i>S. choleraesuis</i>	3.4×10^4	0.5	6.6×10^3	81	0.71
	3.4×10^4	1	3.5×10^2	99	1.99
	3.4×10^4	2	1.1×10^2	99.7	2.49
<i>P. aeruginosa</i>	4.2×10^4	0.5	1.1×10^4	74	0.58
	4.2×10^4	1	3.1×10^1	99.93	3.13
	4.2×10^4	2	1.4×10^1	99.97	3.48

TABLE 7. Qualitative Residual Results at 24 Hours
Following Application of AXEN® 30 ppm

TEST ORGANISM	EXPOSURE TIME ON SLIDE	GROWTH RESULTS FOR LOT 2001-042-001	GROWTH RESULTS FOR LOT 2001-005-001
<i>S. aureus</i>	30 seconds	Growth	Growth
		Growth	Growth
		Extracted (Table 2)	Growth
	1 minute	Growth	Growth
		Growth	Growth
		Extracted (Table 2)	Growth
	2 minutes	Growth	No Growth
		Growth	No Growth
		Extracted (Table 2)	Growth
<i>S. choleraesuis</i>	30 seconds	Growth	Growth
		Growth	Growth
		Extracted (Table 2)	Growth
	1 minute	Growth	Growth
		Growth	Growth
		Extracted (Table 2)	Growth
	2 minutes	Growth	Growth
		Growth	No Growth
		Extracted (Table 2)	Growth

TABLE 7. Qualitative Residual Results at 24 Hours
Following Application of AXEN® 30 ppm (Cont.)

TEST ORGANISM	EXPOSURE TIME ON SLIDE	GROWTH RESULTS FOR LOT 2001-042-001	GROWTH RESULTS FOR LOT 2001-005-001
<i>P. aeruginosa</i>	30 seconds	Growth	Growth
		Growth	Growth
		Extracted (Table 2)	Growth
	1 minute	No Growth	No Growth
		No Growth	Growth
		Extracted (Table 2)	No Growth
	2 minutes	No Growth	No Growth
		No Growth	No Growth
		Extracted (Table 2)	No Growth

TABLE 8. Quantitative Residual Results at 24 Hours
Following Application of AXEN® 30 ppm

TEST ORGANISM	INITIAL COUNTS (CFU/mL)	CONTACT TIME (minutes)	FINAL COUNTS (CFU/mL)	PERCENT (%) REDUCTION	LOG ₁₀ REDUCTION
<i>S. aureus</i>	3.2×10^4	0.5	1.2×10^2	99.6	2.43
	3.2×10^4	1	1.4×10^1	99.96	3.36
	3.2×10^4	2	4	99.99	3.90
<i>S. choleraesuis</i>	3.4×10^4	0.5	5.7×10^3	83	0.78
	3.4×10^4	1	2.5×10^3	93	1.13
	3.4×10^4	2	<1	>99.997	4.53
<i>P. aeruginosa</i>	4.2×10^4	0.5	<1	>99.998	4.62
	4.2×10^4	1	4	99.990	4.02
	4.2×10^4	2	<1	>99.998	4.62

TABLE 9. Results for Neutralization and Growth Promotion

SAMPLE IDENTIFICATION	TEST ORGANISM	CFU/BOTTLE	RESULTS
AXEN® Lot #2001-042-001	<i>S. aureus</i>	62	7/7 Growth
	<i>S. choleraesuis</i>	56	3/3 Growth
	<i>P. aeruginosa</i>	56	10/10 Growth
AXEN® Lot #2001-005-001	<i>S. aureus</i>	62	14/14 Growth
	<i>S. choleraesuis</i>	56	4/4 Growth
	<i>P. aeruginosa</i>	56	20/20 Growth
Media Control	<i>S. aureus</i>	62	1/1 Growth
	<i>S. choleraesuis</i>	56	1/1 Growth
	<i>P. aeruginosa</i>	56	1/1 Growth



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