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FINAL REPORT

STUDY TITLE

AOAC USE DILUTION - CARRIER CONFIRMATION

AUTHOR

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STUDY COMPLETED ON

10 JAN 2002

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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 / 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. 197155

Shelli A. Baxter, B.S. SM(NRM) Nelson Laboratories, Inc.	Study Director Title
Shelli Baxter Signature	11 Jan 2002 Date
Dolana Blount Submitter's Name	Assistant to the President / CEO Title
 Signature	01·23·2002 Date
Dolana Blount Sponsor's Name	Assistant to the President / CEO Title
 Signature	01·23·2002 Date

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)

AOAC USE DILUTION - CARRIER CONFIRMATION

I CERTIFY THAT THE TEST WAS CONDUCTED IN ACCORDANCE

WITH THE USFDA OR USEPA REGULATIONS AS NOTED ABOVE.

LABORATORY NO. 197155

STUDY DIRECTOR: Shelli Baxter DATE: 11 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)

AOAC USE DILUTION - CARRIER CONFIRMATION

Study Director: Final Report Dated:

Shelli A. Baxter, B.S. SM(NRM) 10 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 197155.

2. In accordance with the Good Laboratory Practice Regulations, this study was inspected by the Quality Assurance Unit on: 04 Jan 2002. The findings of the inspection(s) were reported to Management and to the Study Director on: 04 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: Karen M. Schaefer DATE: 15 Jan 2002

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AOAC USE DILUTION - CARRIER CONFIRMATION

LABORATORY NUMBER:	197155
PROTOCOL NUMBER:	200135303-01
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:	21 Dec 2001
SAMPLE RECEIVED DATE:	19 Nov 2001
LAB PHASE START DATE:	03 Jan 2002
LAB PHASE COMPLETION DATE:	10 Jan 2002
REPORT ISSUE DATE:	10 Jan 2002
TOTAL NUMBER OF PAGES:	20

REFERENCES:

AOAC Methods. 1990. 15th Edition. Volume I. Chapter 6, Pages 133-146.

United States Environmental Protection Agency. Office of Pesticide Programs. DIS/TSS-1 Efficacy Data Requirements. Disinfectants for Use on Hard Surfaces. Jan 22, 1982.

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

INTRODUCTION:

This report describes the procedures for the evaluation of AXEN®EPA Registration Number 72977-2 from Innovative Medical Services for hard surface disinfectant efficacy. Two lots of product were tested at 30 parts per million (ppm) against the organisms listed below, following the procedures as outlined in the Association of Official Analytical Chemists (AOAC) Use Dilution Test.

Staphylococcus aureus (MRSA)	ATCC #700698
Enterococcus faecium (VRE)	ATCC #700221
Listeria monocytogenes	ATCC #19111
Escherichia coli OH157	ATCC #43888

PROCEDURES:

CULTURE PREPARATION:

From stock culture, nutrient broth AOAC (NBAOAC) was inoculated with the test organisms and incubated at 37 ± 2°C. The bacteria were transferred at 24 hour intervals for three consecutive days in NBAOAC and then incubated at 37 ± 2°C for 48-54 hours. After incubation, the 48-54 hour test cultures were carefully vortexed for 3-4 seconds and left to stand 10 minutes at room temperature. The cultures were diluted 1:10 using peptone water (PEPW) and to each enough equine serum was added to obtain a 5% total volume organic challenge. Using a sterile nichrome wire, sterile carriers were transferred to the challenge cultures and allowed to remain in contact for 15 minutes.

Carriers were polished stainless steel cylinders 8 mm outside diameter by 6 mm inside diameter by 10 mm long. All dimensions were ± 1 mm. All carriers were type 304 stainless steel (S & L Metal Products). The carriers were removed, shaken to remove excess culture, and placed on end in a vertical position in sterile petri dishes matted with Whatman #2 filter paper. Carriers that fell over in the petri dish were not used and carriers were not allowed to touch each other to prevent improper drying. The carriers were covered and dried at 37 ± 2°C for 40 ± 2 minutes.

TITRATION OF CARRIERS:

For titration of carriers, 10 mL blanks of peptone Tween® (PEPT) solution were prepared. For each organism, two contaminated dried carriers were placed into individual tubes of PEPT which represents the first 1/10 dilution. The tubes were agitated vigorously enough to get bacteria into solution and serial dilutions were made into 9 mL blanks of neutralizer broth (NEUB). The dilution blanks were incubated at 37 ± 2°C. The last tube with growth indicated the $\log_{10}$ titer of organisms on the carrier. AOAC requires carriers to have minimum populations of 1×10^4 colony forming unit (CFU)/carrier.

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:

Each lot of AXEN® was tested in the following way:

Against 10 dried carriers of *S. aureus* ATCC #700698 at 30 seconds, 1 and 2 minutes

Against 10 dried carriers of *E. faecium* ATCC #700221 at 30 seconds, 1 and 2 minutes

Against 10 dried carriers of *L.. monocytogenes* ATCC #19111 at 30 seconds, 1 and 2 minutes

Against 10 dried carriers of *E. coli* OH157 ATCC #43888 at 30 seconds, 1 and 2 minutes

Using sterile glass pipettes, 10 mL aliquots of the disinfectant were placed into sterile test tubes and allowed to equilibrate in a refrigerated waterbath held at 20 ± 0.5°C. Without touching the sides of the test tubes, 1 contaminated dried cylinder was added at 30 second intervals to each tube of disinfectant, swirled three times, and placed back into the waterbath. Following the exposure intervals, the carriers were removed from the disinfectant, shaken to remove residual disinfectant, and transferred to a tube of LETH. The LETH tubes with the carriers were shaken thoroughly. For controls, a dried contaminated carrier for each organism was added to a tube of LETH as a positive control. Uninoculated media tubes served as negative controls. The culture tubes were incubated at 37 ± 2°C for 2 days and scored as (+) or (0) for growth of the challenge organism.

NEUTRALIZATION AND GROWTH PROMOTION:

After incubation, all negative tubes were inoculated with 1-100 CFU of the appropriate organisms to demonstrate neutralization efficacy. To demonstrate growth promotion of the media, the negative control tubes were also inoculated with 1-100 CFU. The inoculating volume was plated in triplicate onto SCDA to verify the inoculating titer. The tubes and plates were incubated at 37 ± 2°C until growth was seen in all tubes.

RESULTS:

The test results are summarized in Tables 1-4. AXEN® 30 ppm solution lot #2001-042-001 demonstrated kill of methicillin resistant *S. aureus* on 1 of 10 carriers after a 30 second exposure, 3 of 10 carriers after a 1 minute exposure, and 10 of 10 carriers after a 2 minute exposure. AXEN® 30 ppm solution lot #2001-005-001 demonstrated kill of methicillin resistant *S. aureus* on 1 of 10 carriers after a 30 second exposure, 3 of 10 carriers after a 1 minute exposure, and 10 of 10 carriers after a 2 minute exposure.

AXEN® 30 ppm solution lot #2001-042-001 demonstrated kill of vancomycin resistant *E. faecium* on 10 of 10 carriers after a 30 second exposure, 9 of 10 carriers after a 1 minute exposure, and 10 of 10 carriers after a 2 minute exposure. AXEN® 30 ppm solution lot #2001-005-001 demonstrated kill of vancomycin resistant *E. faecium* on 8 of 10 carriers after a 30 second exposure, 9 of 10 carriers after a 1 minute exposure, and 10 of 10 carriers after a 2 minute exposure.

AXEN® 30 ppm solution lot #2001-042-001 demonstrated kill of *E.coli* OH157 on 9 of 10 carriers after a 30 second exposure, 10 of 10 carriers after a 1 minute exposure, and 10 of 10 carriers after a 2 minute exposure. AXEN® 30 ppm solution lot #2001-005-001 demonstrated kill of *E. coli* OH157 on 10 of 10 carriers after a 30 second exposure, 9 of 10 carriers after a 1 minute exposure, and 10 of 10 carriers after a 2 minute exposure.

AXEN® 30 ppm solution lot #2001-042-001 demonstrated kill of *L. monocytogenes* on 10 of 10 carriers after a 30 second exposure, 10 of 10 carriers after a 1 minute exposure, and 10 of 10 carriers after a 2 minute exposure. AXEN® 30 ppm solution lot #2001-005-001 demonstrated kill of *L. monocytogenes* on 10 of 10 carriers after a 30 second exposure, 10 of 10 carriers after a 1 minute exposure, and 10 of 10 carriers after a 2 minute exposure.

Carrier titration results can be found in Table 5. All organisms demonstrated at least 1 x 10^4 CFU/carrier.

Neutralization and growth promotion results are summarized in Tables 6-9 . All inoculated tubes were positive for growth after incubation.

Deborah Petric
Technical Reviewer

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

11 Jan 2002
Study Completion Date

SAB/clc

TABLE 1. Disinfectant Efficacy Results
Staphylococcus aureus (MRSA) ATCC #700698

AXEN® LOT NUMBER	TIME POINT	# OF CARRIERS TESTED	# OF CARRIERS EXHIBITING GROWTH	# CARRIERS EXHIBITING NO GROWTH
2001-042-001	30 seconds	10	9	1
	1 minute	10	7	3
	2 minutes	10	0	10
2001-005-001	30 seconds	10	9	1
	1 minute	10	7	3
	2 minutes	10	0	10
Positive Control	N/A	2	2	0
Media Control	N/A	3	0	3

TABLE 2. Disinfectant Efficacy Results
Enterococcus faecium (VRE) ATCC #700221

AXEN® LOT NUMBER	TIME POINT	# OF CARRIERS TESTED	# OF CARRIERS EXHIBITING GROWTH	# CARRIERS EXHIBITING NO GROWTH
2001-042-001	30 seconds	10	0	10
	1 minute	10	1	9
	2 minutes	10	0	10
2001-005-001	30 seconds	10	2	8
	1 minute	10	1	9
	2 minutes	10	0	10
Positive Control	N/A	2	2	0
Media Control	N/A	3	0	3

TABLE 3. Disinfectant Efficacy Results
Escherichia coli OH157 ATCC #43888

AXEN® LOT NUMBER	TIME POINT	# OF CARRIERS TESTED	# OF CARRIERS EXHIBITING GROWTH	# CARRIERS EXHIBITING NO GROWTH
2001-042-001	30 seconds	10	1	9
	1 minute	10	0	10
	2 minutes	10	0	10
2001-005-001	30 seconds	10	0	10
	1 minute	10	1	9
	2 minutes	10	0	10
Positive Control	NA	2	2	0
Media Control	NA	3	0	3

TABLE 4. Disinfectant Efficacy Results
Listeria monocytogenes ATCC #19111

AXEN® LOT NUMBER	TIME POINT	# OF CARRIERS TESTED	# OF CARRIERS EXHIBITING GROWTH	# CARRIERS EXHIBITING NO GROWTH
2001-042-001	30 seconds	10	0	10
	1 minute	10	0	10
	2 minutes	10	0	10
2001-005-001	30 seconds	10	0	10
	1 minute	10	0	10
	2 minutes	10	0	10
Positive Control	NA	2	2	0
Media Control	NA	3	0	3

TABLE 5. Carrier Titration Results

ORGANISM	CARRIER #1	CARRIER #2
S. aureus	10^5 CFU/carrier	10^5 CFU/carrier
E. faecium	10^5 CFU/carrier	10^4 CFU/carrier
E. coli	10^4 CFU/carrier	10^5 CFU/carrier
L. monocytogenes	10^4 CFU/carrier	10^4 CFU/carrier

TABLE 6. Neutralization and Growth Promotion Results
S. aureus

TUBE	EXPOSURE TIME	CFU/TUBE	RESULTS
AXEN® Lot #2001-042-001	30 seconds	32	1/1 (+)
	1 minute	32	3/3 (+)
	2 minutes	32	10/10 (+)
AXEN® Lot #2001-005-001	30 seconds	32	1/1 (+)
	1 minute	32	3/3 (+)
	2 minutes	32	10/10 (+)
Media Control	N/A	32	3/3 (+)

TABLE 7. Neutralization and Growth Promotion Results
E. faecium

TUBE	EXPOSURE TIME	CFU/TUBE	RESULTS
AXEN® Lot #2001-042-001	30 seconds	19	10/10 (+)
	1 minute	19	9/9 (+)
	2 minutes	19	10/10 (+)
AXEN® Lot #2001-005-001	30 seconds	19	8/8 (+)
	1 minute	19	9/9 (+)
	2 minutes	19	10/10 (+)
Media Control	N/A	19	3/3 (+)

TABLE 8. Neutralization and Growth Promotion Results
E. coli

TUBE	EXPOSURE TIME	CFU/TUBE	RESULTS
AXEN® Lot #2001-042-001	30 seconds	28	9/9 (+)
	1 minute	28	10/10 (+)
	2 minutes	28	10/10 (+)
AXEN® Lot #2001-005-001	30 seconds	28	10/10 (+)
	1 minute	28	9/9 (+)
	2 minutes	28	10/10 (+)
Media Control	N/A	28	3/3 (+)

TABLE 9. Neutralization and Growth Promotion Results
L. monocytogenes

TUBE	EXPOSURE TIME	CFU/TUBE	RESULTS
AXEN® Lot #2001-042-001	30 seconds	52	10/10 (+)
	1 minute	52	10/10 (+)
	2 minutes	52	10/10 (+)
AXEN® Lot #2001-005-001	30 seconds	52	10/10 (+)
	1 minute	52	10/10 (+)
	2 minutes	52	10/10 (+)
Media Control	N/A	52	3/3 (+)