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FINAL REPORT

STUDY TITLE

AOAC USE DILUTION - CARRIER CONFIRMATION

AUTHOR

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STUDY COMPLETED ON

09 JAN 2002

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Company: Innovative Medical Services

Company Agent: Dolana Blount

Title: Assistant to the President / CEO

Signature: 

Date: 01.17.02

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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. 194972

Shelli A. Baxter, B.S. SM(NRM) — Study Director
Nelson Laboratories, Inc. — Title

Shelli Baxter — 10 Jan 2002
Signature — Date

Dolana Blount — Assistant to the President / CEO
Submitter's Name — Title

Dolana Blount — 15 Jan 2002
Signature — Date

Dolana Blount — Assistant to the President / CEO
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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)

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. 194972

STUDY DIRECTOR: Shelli Baxter DATE: 10 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) 09 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 194972.

2. In accordance with the Good Laboratory Practice Regulations, this study was inspected by the Quality Assurance Unit on: 19 Oct 2001. The findings of the inspection(s) were reported to Management and to the Study Director on: 19 Oct 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: Sharon M. Schlatter DATE: 11 Jan 2002

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AOAC USE DILUTION - CARRIER CONFIRMATION

LABORATORY NUMBER:	194972
PROTOCOL NUMBER:	200126906-02
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:	12 Oct 2001
SAMPLE RECEIVED DATE:	18 Oct 2001
LAB PHASE START DATE:	18 Oct 2001
LAB PHASE COMPLETION DATE:	08 Jan 2002
REPORT ISSUE DATE:	09 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 AXENOHL® EPA Registration Number 72977-1 from Innovative Medical Services for hard surface disinfectant efficacy. Two lots of product were tested at dilutions of 15 parts per million (ppm), 20 ppm, and 30 ppm against the organisms listed below, following the procedures as outlined in the Association of Official Analytical Chemists (AOAC) Use Dilution Test.

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

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 1:100 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 letheen broth (LETH) medium. 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 CFU/carrier.

SAMPLE PREPARATION

On the day of test, the appropriate dilution of AXENOHL® was prepared by diluting the 2410 ppm concentrate with 5% (w/w) citric acid in purified water. A 15 ppm, 20 ppm, and 30 ppm dilution were prepared and tested.

TEST PERFORMANCE

Each lot of AXENOHL® prepared at 15 ppm was tested in the following way:

Against 10 dried carriers of *S. aureus* ATCC #6538 at 1, 5, and 10 minutes

Against 10 dried carriers of *S. choleraesuis* ATCC #10708 at 1, 5, and 10 minutes

Against 10 dried carriers of *P. aeruginosa* ATCC #15442 at 1, 5, and 10 minutes

Each lot of AXENOHL® prepared at 20 ppm was tested in the following way:

Against 10 dried carriers of *S. aureus* ATCC #6538 at 1, 2, and 5 minutes

Against 10 dried carriers of *S. choleraesuis* ATCC #10708 at 1, 2, and 5 minutes

Against 10 dried carriers of *P. aeruginosa* ATCC #15442 at 1, 2, and 5 minutes

Each lot of AXENOHL® prepared at 30 ppm was tested in the following way:

Against 10 dried carriers of *S. aureus* ATCC #6538 at 30 seconds, 1 and 2 minutes

Against 10 dried carriers of *S. choleraesuis* ATCC #10708 at 30 seconds, 1 and 2 minutes

Against 10 dried carriers of *P. aeruginosa* ATCC #15442 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.

PHENOL RESISTANCE:

The phenol resistance of each test culture was determined according to the following dilutions: *Staphylococcus aureus*: 1:60 and 1:70, *Salmonella choleraesuis*: 1:90 and 1:100 and *Pseudomonas aeruginosa*: 1:80 and 1:90. These dilutions were prepared by diluting a 5% stock solution of phenol (1:20). Sterile test tubes were filled with 5 mL aliquots of the appropriate dilution and allowed to equilibrate in a 20 ± 0.5°C waterbath. At 30 second intervals, 0.5 mL of the challenge culture was added to each tube. The tubes were gently agitated to distribute the culture and replaced into the waterbath. The exposure times were 5, 10 and 15 minutes. After the appropriate exposure time, a loopful from the assay tube was transferred to a tube of LETH. The tubes were incubated at 37 ± 2°C for 2 days and observed for growth of the challenge organisms.

NEUTRALIZATION AND GROWTH PROMOTION

After incubation, all negative tubes were inoculated with 10-100 colony forming units (CFU) of the appropriate organisms to demonstrate neutralization efficacy. To demonstrate growth promotion of the media, the negative control tubes were also inoculated with 10-100 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 1-3. All positive controls showed growth after incubation, all media and negative controls did not show growth.

Carrier titration results can be found in Table 4. All organisms met or exceeded the AOAC required inoculation of at least 1 x 10^4 CFU/carrier.

Neutralization and growth promotion results are summarized in Table 5. All inoculated tubes were positive for growth after incubation.

Phenol resistance results are summarized in Tables 6-8. The AOAC minimal required resistance for each test organism is found in Table 9. All test organisms met or exceeded the AOAC required phenol resistance.

Deborah Petric
Technical Reviewer

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

10 Jan 2002
Study Completion Date

SAB/clc

TABLE 1. Disinfectant Efficacy Results
AXENOHL® 15 ppm Solution

AXENOHL® LOT NUMBER	ORGANISM	TIME POINT	# OF CARRIERS TESTED	# OF CARRIERS SHOWING GROWTH
2001-042-001	*S. aureus*	1 minute	10	1
		5 minutes	10	1
		10 minutes	10	0
	S. choleraesuis	1 minute	10	0
		5 minutes	10	0
		10 minutes	10	0
	P. aeruginosa	1 minute	10	0
		5 minutes	10	0
		10 minutes	10	0
2001-005-001	*S. aureus*	1 minute	10	2
		5 minutes	10	1
		10 minutes	10	0
	S. choleraesuis	1 minute	10	1
		5 minutes	10	0
		10 minutes	10	0
	P. aeruginosa	1 minute	10	0
		5 minutes	10	0
		10 minutes	10	0

TABLE 2. Disinfectant Efficacy Results
AXENOHL® 20 ppm Solution

AXENOHL® LOT NUMBER	ORGANISM	TIME POINT	# OF CARRIERS TESTED	# OF CARRIERS SHOWING GROWTH
2001-042-001	*S. aureus*	1 minute	10	1
		2 minutes	10	0
		5 minutes	10	0
	S. choleraesuis	1 minute	10	0
		2 minutes	10	0
		5 minutes	10	0
	P. aeruginosa	1 minute	10	0
		2 minutes	10	0
		5 minutes	10	0
2001-005-001	*S. aureus*	1 minute	10	0
		2 minutes	10	1
		5 minutes	10	0
	S. choleraesuis	1 minute	10	0
		2 minutes	10	0
		5 minutes	10	0
	P. aeruginosa	1 minute	10	0
		2 minutes	10	0
		5 minutes	10	0

TABLE 3. Disinfectant Efficacy Results
AXENOHL® 30 ppm Solution

AXENOHL® LOT NUMBER	ORGANISM	TIME POINT	# OF CARRIERS TESTED	# OF CARRIERS SHOWING GROWTH
2001-042-001	*S. aureus*	30 seconds	10	0
		1 minute	10	0
		2 minutes	10	0
	S. choleraesuis	30 seconds	10	0
		1 minute	10	0
		2 minutes	10	0
	P. aeruginosa	30 seconds	10	0
		1 minute	10	0
		2 minutes	10	0
2001-005-001	*S. aureus*	30 seconds	10	0
		1 minute	10	0
		2 minutes	10	0
	S. choleraesuis	30 seconds	10	0
		1 minute	10	0
		2 minutes	10	0
	P. aeruginosa	30 seconds	10	0
		1 minute	10	0
		2 minutes	10	0

TABLE 4. Carrier Titration Results

AXENOHL® CONCENTRATION	ORGANISM	CARRIER #1	CARRIER #2
15 ppm	*S. aureus*	10^4 CFU/carrier	10^6 CFU/carrier
	S. choleraesuis	10^6 CFU/carrier	10^6 CFU/carrier
	P. aeruginosa	10^5 CFU/carrier	10^6 CFU/carrier
20 ppm	*S. aureus*	10^5 CFU/carrier	10^5 CFU/carrier
	S. choleraesuis	10^4 CFU/carrier	10^4 CFU/carrier
	P. aeruginosa	10^4 CFU/carrier	10^4 CFU/carrier
30 ppm	*S. aureus*	10^5 CFU/carrier	10^6 CFU/carrier
	S. choleraesuis	10^5 CFU/carrier	10^5 CFU/carrier
	P. aeruginosa	10^4 CFU/carrier	10^5 CFU/carrier

TABLE 5. Neutralization and Growth Promotion Results

AXENOHL® CONCENTRATION	ORGANISM	CFU/TUBE	GROWTH RESULTS
15 ppm	*S. aureus*	80	All tubes positive
	S. choleraesuis	79	All tubes positive
	P. aeruginosa	62	All tubes positive
20 ppm	*S. aureus*	29	All tubes positive
	S. choleraesuis	69	All tubes positive
	P. aeruginosa	34	All tubes positive
30 ppm	*S. aureus*	10	All tubes positive
	S. choleraesuis	49	All tubes positive
	P. aeruginosa	23	All tubes positive

TABLE 6. Phenol Resistance Results
AXENOHL® 15 ppm Solution

EXPOSURE TIME	PHENOL RESISTANCE					
	S. aureus		*P. aeruginosa*		*S. choleraesuis*	
	1:60	1:70	1:80	1:90	1:90	1:100
5 Minutes	Growth	Growth	Growth	Growth	Growth	Growth
10 Minutes	Growth	Growth	Growth	Growth	Growth	Growth
15 Minutes	Growth	Growth	Growth	Growth	Growth	Growth

TABLE 7. Phenol Resistance Results
AXENOHL® 20 ppm Solution

EXPOSURE TIME	PHENOL RESISTANCE					
	S. aureus		*P. aeruginosa*		*S. choleraesuis*	
	1:60	1:70	1:80	1:90	1:90	1:100
5 Minutes	Growth	Growth	Growth	Growth	Growth	Growth
10 Minutes	Growth	Growth	Growth	Growth	Growth	Growth
15 Minutes	No Growth	Growth	Growth	Growth	Growth	Growth

TABLE 8. Phenol Resistance Results
AXENOHL® 30 ppm Solution

EXPOSURE TIME	PHENOL RESISTANCE					
	S. aureus		*P. aeruginosa*		*S. choleraesuis*	
	1:60	1:70	1:80	1:90	1:90	1:100
5 Minutes	Growth	Growth	Growth	Growth	Growth	Growth
10 Minutes	Growth	Growth	Growth	Growth	Growth	Growth
15 Minutes	Growth	Growth	Growth	Growth	Growth	Growth

TABLE 8. AOAC Phenol Resistance Requirements

EXPOSURE TIME	PHENOL RESISTANCE REQUIREMENTS					
	S. aureus		*P. aeruginosa*		*S. choleraesuis*	
	1:60	1:70	1:80	1:90	1:90	1:100
5 Minutes	+ or 0	+	+ or 0	+	+ or 0	+
10 Minutes	+ or 0	+	+ or 0	+	+ or 0	+
15 Minutes	0	+	0	+	0	+ or 0