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FINAL REPORT

MINIMUM INHIBITORY CONCENTRATION (MIC)/ MINIMUM BACTERICIDAL CONCENTRATION (MBC)

PROCEDURE NO. STP0136 REV 01
PROTOCOL DETAIL SHEET NO. 200803431 REV 01

LABORATORY NO. 452819

PREPARED FOR:

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SUBMITTED BY:

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NELSON LABORATORIES

NELSON LABORATORIES, INC.

QAU AUDIT STATEMENT

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

MINIMUM INHIBITORY CONCENTRATION (MIC)/
MINIMUM BACTERICIDAL CONCENTRATION (MBC)

LABORATORY NO. 452819

1. The test was conducted in accordance with the USFDA or USEPA Regulations as noted above.

2. In accordance with the Good Laboratory Practice Regulations, the MIC Test Procedure phase(s) of this study was inspected by the Quality Assurance Unit on: 02 Feb 2009. The findings of the inspection(s) were reported to the Study Director and to Management on: 06 Feb 2009.

3. The Quality Assurance Unit has reviewed this report and has determined that the methods and standard testing procedures are accurately described, and that the reported results accurately reflect the raw data.

4. The name of the study director, the names of other scientists or professionals, and the names of all supervisory personnel, involved in the study:

Michael Neilson
Wellance T. Naeata
Dr. Jerry Nelson
Jeff Hills

QUALITY ASSURANCE: _____ DATE: 16 Feb 2009

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MINIMUM INHIBITORY CONCENTRATION (MIC)/ MINIMUM BACTERICIDAL CONCENTRATION (MBC)

LABORATORY NUMBER:	452819
PROCEDURE NUMBER:	STP0136 REV 01
PROTOCOL DETAIL SHEET NUMBER:	200803431 REV 01
SAMPLE SOURCE:	EnzySurge Ltd.
SAMPLE IDENTIFICATION:	Refer to Tables 1-9
	P.O. #1008
DEVIATIONS:	None
PROTOCOL APPROVAL DATE:	18 Dec 2008
SAMPLE RECEIVED DATE:	26 Nov 2008
LAB PHASE START DATE:	18 Dec 2008
LAB PHASE COMPLETION DATE:	06 Feb 2009
REPORT ISSUE DATE:	13 Feb 2009

INTRODUCTION:

This report describes the procedure for determining the effective use dilution of the sponsor's product against the test organism(s) using a tube dilution method. Serial dilutions were made of the product in bacterial growth media. The test organism(s) were added to the product dilutions and incubated for growth. The dilutions of the product that demonstrated no visible growth of the test organism were plated to confirm lethality of the product.

This procedure is a standard susceptibility assay for antimicrobials and incorporates the intent of the American Society for Mocrobiology (ASM) methodology. Neutralization was confirmed at ≥ 70%.

ACCEPTANCE CRITERIA

All positive controls demonstrated growth of the target organism. All media and negative controls demonstrated no growth of the target organism.

CULTURE PREPARATION:

Tubes of soybean casein digest broth (SCDB) were inoculated with stock cultures of bacteria and incubated at 37 ± 2°C for 18-48 hours.

Where necessary, culture concentrations were adjusted by dilution in 0.9 percent sodium nitrate (NaNO3) provided by sponsor to approximately 10^8 colony forming units (CFU)/mL using visual turbidity. On the day of testing, a standard plate count was performed on the suspension through dilution in 0.9% NaNO3 and plating in triplicate on neutralizer agar (NUAG) to determine the starting titer.

MIC TEST PROCEDURE:

The test product was two-fold serially diluted in sterile purified water (PURW). Next, 5 mL of each product dilution was added to 5 mL of 2X media. The final test dilutions ranged from 1:2 to 1:4096.

Two positive control tubes, per organism, were prepared by mixing 5 mL PURW with 5 mL of 2X media. Two negative control tubes, per sample, were prepared by mixing 5 mL of the lowest product dilution with 5 mL of 2X media. Two media control tubes were prepared by mixing 5 mL PURW with 5 mL of 2X media. No test culture was added to either negative control or media control tubes.

All test sample dilution and positive control tubes were inoculated with 0.05 mL of the test organism. All tubes were incubated at 37 ± 2°C for 16-20 hours. Based upon growth each tube dilution was scored as either positive (+) or negative (0).

MBC TEST PROCEDURE:

Dilutions demonstrating no growth were tested for MBC.

A 0.1 mL aliquot was removed from each tube demonstrating no growth. Each dilution was plated in triplicate on NUAG. For a negative control, sterile 2X media were plated onto NUAG. Positive Controls were made by plating ≤ 100 CFU of the test organisms on NUAG. The test plates were incubated at 37 ± 2°C for 2-4 days.

NEUTRALIZATION VERIFICATION:

The lowest dilution of the test product tested for MLC was tested for neutralization recovery of the test organism in 2X media. 0.1 mL aliquots of the test product were plated in triplicate on NUAG. Three additional plates were prepared for each organism as a titer control.

Plates were spiked with ≤ 100 CFU of the test organism. The plates were incubated at 37 ± 2°C for 2-4 days.

The counts obtained from the titer control were compared to those of the test samples.

RESULTS

MIC Results are summarized in Tables 1-4. Growth is represented as +. No growth is represented as 0.

MBC results are found in Tables 5-8. Values are reported as observed CFU/plate.

Values are considered approximate (~) when plate counts were outside of the statistically accurate range of 25-250 colony forming units (CFU)/plate for bacteria. Less than symbols (<) are applied to recovery values where no CFU were observed on the plates. This denotes the limit of detection for the test.

Neutralization results are found in Table 9.

Testing met the acceptance criteria previously stated in this report.

CONCLUSION:

Interpretation of the data is the responsibility of the sponsor and no conclusion can be made by Nelson Laboratories, Inc. (NLI).

DATA DISPOSITION:

The raw data and final report from this study are archived at NLI or an approved off-site location.

STATEMENT OF UNCERTAINTY

If applicable, a statement of uncertainty is available to sponsors upon request.

Wellance T. Naeata, B.S.
Study Director

16 Feb 2009
Study Completion Date

jzw

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EnzySurge Ltd.
Lab Number 452819

Minimum Inhibitory Concentration (MIC)/
Minimum Bactericidal Concentration (MBC)

TABLE 1. MIC Results
Sample Identification: Silver Stream Lot #SIL-001271008
Silver Buffered Solution 250ml
Active ingrediant: Each ml contains Silver Nitrate 0.1mg
Organism Identification: *Eschericha coli* ATCC #8739

DILUTION (MOLAR)	GROWTH +/0		
	REP 1	REP 2	REP 3
1:2	0	0	0
1:4	0	0	0
1:8	0	0	0
1:16	+	+	+
1:32	+	+	+
1:64	+	+	+
1:128	+	+	+
1:256	+	+	+
1:512	+	+	+
1:1024	+	+	+
1:2048	+	+	+
1:4096	+	+	+
Positive Control	+	+	+
Negative Control	0	0	0
Media Control	0	0	0

MIC: 1:8

Titer: 1.7×10^8 CFU/mL

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EnzySurge Ltd.
Lab Number 452819

Minimum Inhibitory Concentration (MIC)/
Minimum Bactericidal Concentration (MBC)

TABLE 2. MIC Results
Sample Identification: Silver Stream Lot #SIL-001271008
Silver Buffered Solution 250ml
Active ingrediant: Each ml contains Silver Nitrate 0.1mg
Organism Identification: *Staphylococcus epidermidis* ATCC #12228

DILUTION (MOLAR)	GROWTH +/0		
	REP 1	REP 2	REP 3
1:2	0	0	0
1:4	0	0	0
1:8	0	0	0
1:16	+	+	+
1:32	+	+	+
1:64	+	+	+
1:128	+	+	+
1:256	+	+	+
1:512	+	+	+
1:1024	+	+	+
1:2048	+	+	+
1:4096	+	+	+
Positive Control	+	+	+
Negative Control	0	0	0
Media Control	0	0	0

MIC: 1:8

Titer: 5.7×10^7 CFU/mL

TABLE 3. MIC Results
Sample Identification: Silver Stream Lot #SIL-001271008
Silver Buffered Solution 250ml
Active ingrediant: Each ml contains Silver Nitrate 0.1mg
Organism Identification: *Klebsiella pneumoniae* ATCC #4352

DILUTION (MOLAR)	GROWTH +/0		
	REP 1	REP 2	REP 3
1:2	0	0	0
1:4	0	0	0
1:8	0	0	0
1:16	+	+	+
1:32	+	+	+
1:64	+	+	+
1:128	+	+	+
1:256	+	+	+
1:512	+	+	+
1:1024	+	+	+
1:2048	+	+	+
1:4096	+	+	+
Positive Control	+	+	+
Negative Control	0	0	0
Media Control	0	0	0

MIC: 1:8

Titer: 1.3×10^8 CFU/mL

TABLE 4. MIC Results
Sample Identification: Silver Stream Lot #SIL-001271008
Silver Buffered Solution 250ml
Active ingrediant: Each ml contains Silver Nitrate 0.1mg
Organism Identification: *Staphylococcus aureus* ATCC #6538

DILUTION (MOLAR)	GROWTH +/0		
	REP 1	REP 2	REP 3
1:2	0	0	0
1:4	0	0	0
1:8	+	+	+
1:16	+	+	+
1:32	+	+	+
1:64	+	+	+
1:128	+	+	+
1:256	+	+	+
1:512	+	+	+
1:1024	+	+	+
1:2048	+	+	+
1:4096	+	+	+
Positive Control	+	+	+
Negative Control	0	0	0
Media Control	0	0	0

MIC: 1:4

Titer: 1.4×10^8 CFU/mL

TABLE 5. MBC Plate Counts
Sample Identification: Silver Stream Lot #SIL-001271008
Silver Buffered Solution 250ml
Active ingrediant: Each ml contains Silver Nitrate 0.1mg
Organism Identification: *Eschericha coli* ATCC #8739

REPLICATE	PLATE #	SAMPLE DILUTION		
		1:2	1:4	1:8
1	1	0	0	~534
	2	0	0	~604
	3	0	0	~232
2	1	0	0	~768
	2	0	0	~928
	3	0	0	~444
3	1	0	0	~916
	2	0	0	~696
	3	0	0	~528

MBC: 1:4

~ = Approximately

TABLE 6. MBC Plate Counts
Sample Identification: Silver Stream Lot #SIL-001271008
Silver Buffered Solution 250ml
Active ingrediant: Each ml contains Silver Nitrate 0.1mg
Organism Identification: *Staphylococcus epidermidis* ATCC #12228

REPLICATE	PLATE #	SAMPLE DILUTION		
		1:2	1:4	1:8
1	1	7	111	TNTC
	2	10	126	TNTC
	3	8	119	TNTC
2	1	16	112	TNTC
	2	14	93	TNTC
	3	10	109	TNTC
3	1	16	113	TNTC
	2	16	179	TNTC
	3	15	160	TNTC

MBC: Unable to Determine

TNTC = Too numerous to count

TABLE 7. MBC Plate Counts
Sample Identification: Silver Stream Lot #SIL-001271008
Silver Buffered Solution 250ml
Active ingrediant: Each ml contains Silver Nitrate 0.1mg
Organism Identification: *Klebsiella pneumoniae* ATCC #4352

REPLICATE	PLATE #	SAMPLE DILUTION		
		1:2	1:4	1:8
1	1	0	0	7
	2	0	0	11
	3	0	0	15
2	1	0	0	23
	2	0	0	18
	3	0	0	21
3	1	0	0	1
	2	0	0	4
	3	0	0	2

MBC: 1:4

TABLE 8. MBC Plate Counts
Sample Identification: Silver Stream Lot #SIL-001271008
Silver Buffered Solution 250ml
Active ingrediant: Each ml contains Silver Nitrate 0.1mg
Organism Identification: *Staphylococcus aureus* ATCC #6538

REPLICATE	PLATE #	SAMPLE DILUTION	
		1:2	1:4
1	1	255	TNTC
	2	234	TNTC
	3	256	TNTC
2	1	384	TNTC
	2	422	TNTC
	3	386	TNTC
3	1	368	TNTC
	2	390	TNTC
	3	363	TNTC

MBC: Unable to Determine

TNTC = Too numerous to count

TABLE 9. Neutralization
Sample Identification: Silver Stream Lot #SIL-001271008
Silver Buffered Solution 250ml
Active ingrediant: Each ml contains Silver Nitrate 0.1mg

SAMPLE DILUTION	ORGANISM IDENTIFICATION	PRECENT NEUTRALIZATION RECOVERY
1:2	*Eschericha coli* ATCC #8739	83
1:2	*Staphylococcus epidermidis* ATCC #12228	117
1:2	*Klebsiella pneumoniae* ATCC #4352	117
1:2	*Staphylococcus aureus* ATCC #6538	100

NELSON	FORM TITLE: PDS Approval Form	PDS NUMBER: 200803431
		PDS REVISION: 1

PREPARED FOR SPONSOR		LABORATORY / CONTRACTOR
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EMAIL:	noacohen@post.tau.ac.il	
PHONE:	011-972-547938830	
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PROTOCOL SPECIFICATIONS			
PARENTAL DOCUMENT:	Minimum Inhibitory Concentration -MIC /Minimum Bactericidal Concentration -MBC, STP0138, 1		
SECTION:	Pharmaceuticals		
PDS INITIATION DATE:	15-Dec-2008	EXPIRATION DATE:	15-Dec-2010

JUSTIFICATION:

Per sponsors request.

PROTOCOL SPECIFICATIONS:

Perform procedures applicable to MIC testing. Perform sections 12-13 only if approved by sponsor after sample/s show inhibition from the MIC test.

1. Replicate: Triplicate.
2. Organisms:
   Escherichia coli ATCC #8739
   Pseudomonas aeruginosa ATCC #9027
   Staphylococcus aureus ATCC #6538
   Candida albicans ATCC #10231
   Aspergillus niger ATCC #16404
3. Test 12 dilutions using a 1:2 dilution scheme.
4. Use 0.9% Sodium nitrate (NaNO3) instead of Physiological saline (PHSS) for all organism preparations

○ Additional pages attached for protocol specifications ◉ No additional pages needed

The sponsor is responsible for test/control article characterization.
This includes, but is not limited to, identity, strength, purity, and stability.
PLEASE SIGN, DATE, & RETURN TO NELSON LABORATORIES

SPONSOR APPROVAL:

*SIGNATURE: [signature]

DATE: 12.12.08

PRINT NAME: Carmit Cplin

NELSON LABS STUDY DIRECTOR APPROVAL:

SIGNATURE: [signature]

DATE: 18 Dec 2008

PRINT NAME: W. TONI NAEATA

*SIGNING THIS DOCUMENT SIGNIFIES AN ACCEPTANCE OF THE NELSON LABORATORIES TESTING TERMS AND CONDITIONS AS ATTACHED OR AS LISTED AT WWW.NELSONLABS.COM/PROTOCOLCONDITIONS.ISP

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SAMPLE ID.	See sample submission form		

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