Nelson Labs™
A Sotera Health company

Sponsor:
Daniel Albahari
EnzySurge Ltd.
Shabazi 26 St.
Rosh Ha'Ayin, IL 48021
ISRAEL

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

Test Article:	Batch #SSG241017
Purchase Order:	1728
Study Number:	1006374-S01
Study Received Date:	30 Nov 2017
Testing Facility:	Nelson Laboratories, LLC 6280 S. Redwood Rd. Salt Lake City, UT 84123 U.S.A.
Test Procedure(s):	Standard Test Protocol (STP) Number: STP0136 Rev 01 Customer Specification Sheet (CSS) Number: 201707791 Rev 01
Deviation(s):	None

Summary: 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 Microbiology (ASM) methodology. All test method acceptance criteria were met.

Results:

Organism	Inoculum Titer (CFU/mL)	MIC Results	MBC Results
Escherichia coli, ATCC #8739	1.1×10^8	1:32	1:4
Staphylococcus epidermidis, ATCC #12228	1.0×10^8	1:32	None*
Klebsiella pneumoniae, ATCC #4352	4.6×10^8	1:32	1:8
Staphylococcus aureus, ATCC #6538	1.3×10^8	1:16	None*

* - Minimum bactericidal concentration was not obtained at the concentrations tested.

Study Director — Wellance T. Naeata, B.S.

16 MAR 2018
Study Completion Date

1006374-S01

Neutralization:

Organism	Test Article Dilution	Percent Neutralization Recovery
Escherichia coli, ATCC #8739	2XMHBR	83%
Staphylococcus epidermidis, ATCC #12228	2XMHBR	100%
Klebsiella pneumoniae, ATCC #4352	2XMHBR	110%
Staphylococcus aureus, ATCC #6538	2XMHBR	84%

Test Method Acceptance Criteria: All positive controls demonstrated growth of the target organism. All media and negative controls demonstrated no growth of the target organism. Neutralization was confirmed at ≥ 70%.

Culture Preparation: Tubes of Mueller-hinton broth (MHBR) 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 Purified water (PURW) to approximately 10^8 CFU/mL using visual turbidity. On the day of testing, a standard plate count was performed on the suspension through dilution in PURW and plating in triplicate on neutralizer agar (NUAG) to determine the starting titer.

Procedure:
MIC Test: 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 test article, 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 article 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: 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 MBC was tested for neutralization recovery of the test organism in 2X media. Aliquots of the test product (0.1 mL) 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 articles.

Quality Assurance Statement

Compliance Statement: The test was conducted in accordance with the USFDA (21 CFR Parts 58, 210, 211, and 820) Regulations. This final report reflects the raw data.

Activity	Date
Study Initiation	15 Jan 2018
Phase Inspected by Quality Assurance: MIC Test Procedure	07 Mar 2018
Audit Results Reported to Study Director	13 Mar 2018
Audit Results Reported to Management	14 Mar 2018

Scientists	Title
Thomas Pace	Supervisor
Wellance Naeata	Study Director

Data Disposition: The study plan, raw data and final report from this study are archived at Nelson Laboratories, LLC or an approved off-site location.

Quality Assurance

16 Mar 2018
Date