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FINAL REPORT

TIME KILL STUDY

PROCEDURE NO. STP0158 REV 01
PROTOCOL DETAIL SHEET NO. 200901442 REV 01

LABORATORY NO. 474527A

PREPARED FOR:

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QAU AUDIT STATEMENT

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

TIME KILL STUDY

LABORATORY NO. 474527A

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 Culture Prep phase(s) of this study was inspected by the Quality Assurance Unit on: 13 May 2009. The findings of the inspection(s) were reported to the Study Director and to Management on: 09 Jun 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:

Mike Neilson
Peter Croci
Dr. Jerry Nelson
Jeff Hills

QUALITY ASSURANCE: Rut Mtz DATE: 16 Jun 2009

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TIME KILL STUDY

LABORATORY NUMBER:	474527A
PROCEDURE NUMBER:	STP0158 REV 01
PROTOCOL DETAIL SHEET NUMBER:	200901442 REV 01
SAMPLE SOURCE:	American Biotech Labs
SAMPLE IDENTIFICATION:	Refer to Tables 1-2
DEVIATIONS:	None
PROTOCOL APPROVAL DATE:	08 May 2009
SAMPLE RECEIVED DATE:	07 May 2009
LAB PHASE START DATE:	08 May 2009
LAB PHASE COMPLETION DATE:	09 Jun 2009
REPORT ISSUE DATE:	11 Jun 2009

INTRODUCTION:

This report describes the procedure for the evaluation of products for anti-microbial activity against selected organisms at representative contact times. Products are evaluated in a liquid matrix. The test organisms and contact times are chosen by the sponsor. This is a quantitative test that allows the determination of the amount of organism reduction at pre-determined intervals.

ACCEPTANCE CRITERIA:

Negative controls must be negative for growth. Positive controls must be positive for growth. Neutralization must be confirmed at ≥ 70%. Specific criteria for pass/fail of the sample must be determined by the Sponsor.

INOCULUM PREPARATION:

Plates of soybean casein digest agar (SCDA) media were inoculated with stock cultures of bacteria and incubated at 30-35°C for 18-24 hours. Growth was harvested from the surface using a sterile bent glass rod and purified water solution (PURW).

Where necessary, culture suspensions were adjusted for the test procedure with purified water solution (PURW) to approximately (~) 10^8 colony forming units (CFU)/mL using visual turbidity.

SAMPLE PREPARATION:

Samples were prepared according to the product label or sponsor instructions and were tested without any additional manipulation or dilution.

NEUTRALIZATION:

A 1 mL aliquot of the test sample was mixed with 9 mL of Letheen broth (LETH). An additional tube of 10 mL of LETH was prepared as a titer control. The tubes were inoculated with 0.1 mL of a test organism suspension diluted to approximately ≤10,000 CFU/mL, then mixed thoroughly. Aliquots from each tube were plated in triplicate onto SCDA and incubated as described in the test procedure.

The neutralizer is considered effective for the product if the number of CFU on the test plates is ≥ 70% of the control plates.

CONTROLS:

Positive control tubes containing 10 mL physiological saline (PHSS) were prepared. 0.1 mL of the test organism was added to each tube. The positive control was assayed at 0 and 24 hour.

The negative control consisted of plating sterile aliquots of applicable liquid media in triplicate and incubating as described in the test procedure.

TEST PROCEDURE:

Tubes containing 10 mL of each test sample were prepared and inoculated with 0.1 mL of the test organism to yield ~10^6 CFU/mL. The samples were mixed thoroughly.

At 1 hour and 24 hours of exposure 1.0 mL aliquots of the test suspension were removed and added to 9 mL of neutralizer. The tubes were mixed thoroughly. Serial dilutions were made in the appropriate neutralizer and assayed using a standard spread plate method.

All plating was performed in triplicate. Bacterial samples were plated onto SCDA and incubated at 30-35°C for 2-5 days.

CALCULATIONS:

The log reduction values were calculated using the following formula:

$$\log reduction = \log U - \log C$$

Where U = Average positive control titer
Where C = Average recovered counts

The percent reduction values were calculated using the following formula:

$$\% \, reduction = 1 - \frac{1}{10^{(\log \text{ reduction})}} \text{x } 100\%$$

The percent neutralization is obtained according to the following equation:

$$\% \, Neutralization = \frac{Average \; Sample \; Counts/Plate}{Average \; Control \; Counts/Plate}$$

RESULTS:

All testing was performed in accordance with Nelson Laboratories, Inc. (NLI) protocol detail sheet #200901442 REV 01.

The average sample and control counts, percent reductions and $\log_{10}$ reductions for the samples can be found in Tables 1. Values are considered approximate (~) when plate counts were outside of the statistically accurate range of 25-250 CFU/plate for bacteria and yeast and 8-80 CFU/plate for mold. 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 summarized in Table 2 with all samples demonstrating ≥ 70% recovery.

Testing met the acceptance criteria previously described 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.

Peter Croci, B.A.
Study Director

11 Jun 2009
Study Completion Date

PC/es

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American Biotech Labs
Lab Number 474527A

Time Kill Study

TABLE 1. Results
Sample Identification: ASAP Wound Dressing Gel 050509-1

ORGANISM	EXPOSURE INTERVALS	AVERAGE CONTROL TITER (CFU/mL)	PERCENT REDUCTION (%)	LOG_{10} REDUCTION
Staphylococcus aureus, ATCC #6538	1 Hour	2.3×10^6	>99.99914	>5.06
	24 Hour	2.1×10^6	>99.99905	>5.02
	24 Hour Control	2.1×10^6	11	0.05

TABLE 2. Neutralization

SAMPLE IDENTIFICATION	ORGANISM	AVERAGE CONTROL COUNTS (CFU)	AVERAGE SAMPLE COUNTS (CFU)	PERCENT NEUTRALIZATION (%)
ASAP Wound Dressing Gel 050509-1	*S. aureus*	39	44	113

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