NELSON LABORATORIES

Sponsor:
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Agar Overlay GLP Report

Test Article: ASAP 10 Silver Solution Lot 13112
Laboratory Number: 709563
Study Received Date: 27 Aug 2013
Test Procedure(s): Standard Test Protocol (STP) Number: STP0031 Rev 08
Protocol Detail Sheet (PDS) Number: 201304211 Rev 01

Summary: The Agar Overlay test was designed to determine the cytotoxicity of diffusible components from materials or solutions. A layer of agar was added over a cell monolayer to act as a cushion to protect the cells from mechanical damage while allowing the diffusion of leachable materials. The test articles were then placed on top of the agar layer and incubated. The cell monolayers were examined and scored based on the degree of cellular destruction. All test method acceptance criteria were met. The test procedure(s) listed above were followed without deviation.

Results:
Test Article:

Results Pass/Fail	Scores				Amount Tested
	#1	#2	#3	Average	
Pass	0	1	1	1	≥ 100 mm^2 per well

Controls:

Identification	Scores				Amount Tested
	#1	#2	#3	Average	
Negative Control – Polypropylene Pellets	0	0	0	0	≥ 100 mm^2 per well
Positive Control – Latex Natural Rubber	4	4	4	4	≥ 100 mm^2 per well

Acceptance Criteria: The United States Pharmacopeia & National Formulary (USP <87>) states that the test article meets the requirements if the reactivity grade is not greater than grade 2 or a mild reactivity. The ANSI/AAMI/ISO 10993-5 standard states that the achievement of a numerical grade greater than 2 is considered a cytotoxic effect. Nelson Laboratories acceptance criteria was based upon the negative control receiving "0" reactivity grades and positive control receiving 3-4 reactivity grades (moderate to severe).

ACLASS ACCREDITED TESTING

Study Director Chad Summers, A.S., ASQ CQA

10 Sep 2013
Study Completion Date

Procedure: Six well cell culture plates were seeded with a verified quantity of industry standard L-929 cells (ATCC CCL-1) and incubated at 37 ± 1°C with 5 ± 1% CO_2 until approximately 80% confluent. The agar overlay consisted of an equal mixture of 1NAGAR (1.0%) and 2X MEM + 10% bovine calf serum. Liquid or gel test articles were applied to sterile filter discs testing no less than 0.1 mL per well. Positive and negative reference controls were included with each assay.

All tests were performed using three test wells per test article. After the addition of the test articles, the cell culture plates were incubated as described above for 24-26 hours. Following incubation, cells were evaluated microscopically using the evaluation criteria outline below:

Grade	Description Of Zone
0	No detectable zone around or under the test article.
1	Some malformed or degenerate cells under the test article.
2	Zone limited to area under the test article and less than 0.45 cm beyond the test article.
3	Zone extends 0.45 to 1.0 cm beyond the test article.
4	Zone extends greater than 1 cm beyond the test article.

The results from the three wells were averaged to give an average cytotoxicity score.

Quality Assurance Statement

Compliance Statement: The test was conducted in accordance with the USFDA (21 CFR Part 58) Regulations. This final report reflects the raw data.

Activity	Date
Study Initiation	04 Sep 2013
Phase Inspected by Quality Assurance: Interpretation	05 Sep 2013
Audit Results Reported to Study Director	06 Sep 2013
Audit Results Reported to Management	06 Sep 2013

Scientists	Title
Michelle Lee	Supervisor
Chad Summers	Study Director

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

Quality Assurance

10 SEP 2013
Date