NELSON LABORATORIES

Sponsor:
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MEM Elution GLP Report

Test Article: 1) DLE-14-48-1
2) Buffer Control Solution
Purchase Order: EI600881
Study Number: 864525-S01
Study Received Date: 21 Dec 2015
Test Procedure(s): Standard Test Protocol (STP) Number: STP0032 Rev 09
Protocol Detail Sheet (PDS) Number: 201504884 Rev 01

Summary: The Minimal Essential Media (MEM) Elution test was designed to determine the cytotoxicity of extractable substances. The test article was added to cell monolayers 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:

Identification	Dilution	Results Pass/Fail	Scores			
			#1	#2	#3	Average
Test Article 1	Full Strength	**Fail**	3	3	3	3
	1:2	**Pass**	2	2	2	2
	1:4	**Pass**	1	1	1	1
	1:8	**Pass**	1	1	1	1
	1:16	**Pass**	1	1	1	1
Test Article 2	Full Strength	**Pass**	2	2	2	2
	1:2	**Pass**	1	1	1	1
	1:4	**Pass**	0	0	0	0
	1:8	**Pass**	0	0	0	0
	1:16	**Pass**	0	0	0	0

Bobbi Rushton Castro
Study Director Bobbi L. Rushton-Castro

ANAB ACCREDITED TESTING LABORATORY
04 Jan 2016
Study Completion Date

Controls:

Identification	Scores				Extraction Ratio	Amount Tested / Extraction Solvent Amount
	#1	#2	#3	Average		
Negative Control - Polypropylene Pellets	0	0	0	0	0.2 g/mL	4 g / 20 mL
Media Control	0	0	0	0	N/A	20 mL
Positive Control - Latex Natural Rubber	4	4	4	4	0.2 g/mL	4 g / 20 mL

Acceptance Criteria: The United States Pharmacopeia & National Formulary (USP <87>) states that the test article meets the requirements, or receives a passing score (**Pass**) 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, or a failing score (**Fail**).

Nelson Laboratories acceptance criteria was based upon the negative and media controls receiving "0" reactivity grades and positive controls receiving a 3-4 reactivity grades (moderate to severe). The test was considered valid as the control results were within acceptable parameters.

The cell monolayers were examined microscopically. The wells were scored as to the degree of discernable morphological cytotoxicity on a relative scale of 0 to 4:

Conditions of All Cultures	Reactivity	Grade
No cell lysis, intracytoplasmic granules.	None	0
Less than or equal to 20% rounding, occasional lysed cells.	Slight	1
Greater than 20% to less than or equal to 50% rounding, no extensive cell lysis.	Mild	2
Greater than 50% to less than 70% rounding and lysed cells.	Moderate	3
Nearly complete destruction of the cell layers.	Severe	4

The results from the three wells were averaged to give a final cytotoxicity score.

Procedure: The liquid test articles were tested by combining 16 mL of test article with a 4 mL aliquot of 5X MEM + 50% bovine calf serum. The pH was adjusted with 7.5% Sodium Bicarbonate. Multiple well cell culture plates were seeded with a verified quantity of industry standard L-929 cells (ATCC CCL-1) and incubated until approximately 80% confluent. The test articles and control extracts were added to the cell monolayers in triplicate. The cells were incubated at 37 ± 1°C with 5 ± 1% CO_2 for 48 ± 3 hours.

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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	28 Dec 2015
Phase Inspected by Quality Assurance: Cell Exposure	31 Dec 2015
Audit Results Reported to Study Director	03 Jan 2016
Audit Results Reported to Management	04 Jan 2016

Scientists	Title
Chad Summers	Supervisor
Bobbi Rushton-Castro	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

04 Jan 2016
Date

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