NELSON LABORATORIES

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Agar Overlay Final Report

Test Article:	Dartex product PER406C-1346 Black on Jade, Pro Incon, batch 705-608
Purchase Order:	16260
Study Number:	972081-S01
Study Received Date:	21 Jun 2017
Testing Facility:	Nelson Laboratories, LLC, a Business Unit of Sterigenics International 6280 S. Redwood Rd. Salt Lake City, UT 84123 U.S.A.
Test Procedure(s):	Standard Test Protocol (STP) Number: 801-STP0031 Rev 08

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. Testing was performed in compliance with US FDA good manufacturing practice (GMP) regulations 21 CFR Parts 210, 211 and 820. 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	0	0	0	≥ 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

ANAB ACCREDITED ISO/IEC 17025 TESTING LABORATORY

Danielle Short for
Study Director — McKenna Wild, B.S.

28 Jun 2017
Study Completion Date

Test Method 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).

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 1% noble agar and 2X Minimal Essential Media + 10% bovine calf serum. Solid test articles were placed directly on the solidified agar overlay testing ≥ 100 mm^2 per test 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.