Nelson Labs
A Sotera Health company

Sponsor:
Breanna All
Parson Adhesives, Inc.
2545 Eastside Park Rd
Evansville, IN 47715

Agar Overlay Final Report

Test Article:	1. Parlite 4790 2. Parlite 4035
Purchase Order:	25191
Study Number:	1475917-S01
Study Received Date:	15 Dec 2021
Test Started Date:	20 Dec 2021
Test Finished Date:	21 Dec 2021
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: STP0031 Rev 09
Deviation(s):	None

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.

Results:

Test Article:

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

Brittany Love electronically approved for — 23 Dec 2021 19:17 (+00:00)
Study Director — Danielle Short — Study Completion Date and Time

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 for no less than 24 hours, until confluency approaches 80-90%. The agar overlay consisted of an equal mixture of 1% noble agar and 2X Minimal Essential Media + 10% bovine serum. Solid test articles were placed directly on the solidified agar overlay testing no less than 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	Reactivity	Description of Zone
0	None	No detectable zone around or under the test article.
1	Slight	Some malformed or degenerate cells under the test article.
2	Mild	Zone limited to area under the test article and less than 0.45 cm beyond the test article.
3	Moderate	Zone extends 0.45 to 1.0 cm beyond the test article.
4	Severe	Zone extends greater than 1 cm beyond the test article.

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

References:

USP 41-NF 36. 2018. <87> *Biological Reactivity Tests, In Vitro*. The United States Pharmacopeial Convention, Rockville, MD. (CRD287)

USP 41-NF 36. 2018. <1031> *The Biocompatibility of Materials Used in Drug containers, Medical Devices, and Implants*. The United States Pharmacopeial Convention, Rockville, MD. (CRD288)

ISO 10993-5:2009/(R)2017. *Biological Evaluation of Medical Devices - Part 5: Tests for cytotoxicity, in-vitro methods*. International Organization for Standardization, Geneva, Switzerland. (CRD025)

ISO 10993-12:2021. *Biological Evaluation of Medical Devices - Part 12: Sample preparation and reference materials*. International Organization for Standardization, Geneva, Switzerland. (CRD023)

ISO 10993-1:2018. *Biological Evaluation of Medical Devices - Part 1: Evaluation and testing within a risk management process*. International Organization for Standardization, Geneva, Switzerland. (CRD027)