NELSON LABORATORIES

Agar Overlay Final Report

Test Article: Platinum Bond, lot number 90460J
Purchase Order: 11825
Laboratory Number: 598075A
Study Received Date: 06 Sep 2011
Test Procedure(s): Standard Test Protocol (STP) Number: STP0031 Rev 07

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.

Results:

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

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 2X agar (1.0%) and 2X MEM + 10% bovine calf serum. Solid test articles were placed directly on the solidified agar overlay testing ≥ 100 mm^2 per test well. Liquid or gel test articles were applied to sterile filter discs testing no less than 0.1 mL per well. Powders, resins, or irregular materials were placed directly onto the solidified agar, testing no less than 100 mg per well. Positive and negative reference controls were included with each assay.

Technical Reviewer

ACLASS ACCREDITED TESTING

Study Director Christine Jensen

13 Sep 2011
Study Completion Date

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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.
3	Zone extends to 1.0 cm beyond the test article.
4	Zone greater than 1 cm in extension from test article.

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