

REPORT

TEST FACILITY

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CONFIDENTIAL

STUDY TITLE

Cytotoxicity Study Using the ISO Agarose Overlay Method

TEST ARTICLE NAME

M139 Pressure Sensitive Adhesive

TEST ARTICLE IDENTIFICATION

1592762 D794948420001

NAMSA

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Summary

The test article, M139 Pressure Sensitive Adhesive, was evaluated to determine the potential for cytotoxicity. This study was conducted based on the requirements of ISO 10993-5, Biological evaluation of medical devices - Part 5: Tests for *in vitro* cytotoxicity. Triplicate wells were dosed with a 1 cm x 1 cm portion of the test article. Triplicate wells were dosed with a 1 cm length portion of high density polyethylene as a negative control. Triplicate wells were dosed with a 1 cm x 1 cm portion of latex as a positive control. Each was placed on an agarose surface directly overlaying a subconfluent monolayer of L-929 mouse fibroblast cells. After incubating at 37°C in the presence of 5% CO₂ for 24-26 hours, the cultures were examined macroscopically and microscopically for any abnormal cell morphology and cell lysis.

The test article showed no evidence of causing any cell lysis or toxicity. The test article met the requirements of the test since the grade was less than or equal to a grade 2 (mild reactivity).

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1. Introduction

1.1 Purpose

The purpose of this study was to determine the potential of a test article to cause cytotoxicity.

1.2 Testing Guidelines

This study was based on the requirements of the International Organization for Standardization 10993-5, Biological evaluation of medical devices - Part 5: Tests for *in vitro* cytotoxicity.

This test was performed in compliance with the ISO 13485 standard, with the test method accredited to the ISO 17025 standard.

1.3 Dates

Test Article Received:	May 30, 2018
Cells Dosed:	May 31, 2018
Observations Concluded:	June 1, 2018

2. Identification of Test and Control Articles

The test article provided by the sponsor was identified and handled as described below:

Table 1: Test Article

Name:	M139 Pressure Sensitive Adhesive
Identification:	1592762 D794948420001
Physical Description of the Test Article:	M139 adhesive on foam, covered with white paper release liner.
Storage Conditions:	Room Temperature

Table 2: Negative Control Article

Name:	High density polyethylene (HDPE)
Strength, Purity, Composition or Other Characteristics:	Composition: polyethylene

Table 3: Positive Control Article

Name:	Powder-free latex gloves
Strength, Purity, Composition or Other Characteristics:	Composition: natural rubber latex, zinc carbamate accelerators, zinc oxide, and titanium dioxide

Table 4: Ancillary Materials

Growth Media:	Single strength Minimum Essential Medium supplemented with 5% fetal bovine serum, 2% antibiotics (100 units/mL penicillin, 100 µg/mL streptomycin, and 2.5 µg/mL amphotericin B) and 1% (2 mM) L-glutamine (1X MEM) Double strength Minimum Essential Medium supplemented with 10% fetal bovine serum, 4% antibiotics (200 units/mL penicillin, 200 µg/mL streptomycin and 5.0 µg/mL amphotericin B) and 2% (4 mM) L-glutamine (2X MEM)
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3. Test System

3.1 Test System and Justification

Mammalian cell culture monolayer consisting of L-929 mouse fibroblast cells free from mycoplasma (ECACC Catalog No. 85103115) was used. *In vitro* mammalian cell culture studies have been used historically to evaluate cytotoxicity of biomaterials and medical devices.

3.2 Test System Management

L-929 mouse fibroblast cells were propagated and maintained in flasks containing 1X MEM at 37°C with 5% carbon dioxide (CO₂). For this study, cells were seeded in 10 cm² cell culture wells, labeled with passage number and date, and incubated at 37°C in the presence of 5% CO₂ to obtain subconfluent monolayers of cells prior to use. Aseptic procedures were used in the handling of the cell cultures following approved NAMSA Standard Operating Procedures.

3.3 Preparation of Agarose Overlay

The culture wells were selected which contained a subconfluent cell monolayer. The agarose mixture was prepared with equal amounts of 2% agarose and 2X MEM supplemented with neutral red. The growth medium in each well was replaced with 2.0 mL of the agarose mixture. The agarose mixture was allowed to solidify over the cells to form the agarose overlay.

4. Method

4.1 Test Article Preparation

The paper liner was removed prior to dosing. The adhesive side was placed against the agarose for testing. Triplicate wells were dosed with a 1 cm x 1 cm portion of the test article.

4.2 Control Article Preparation

Negative Control: Triplicate wells were dosed with a 1 cm length portion of HDPE.

Positive Control: Triplicate wells were dosed with a 1 cm x 1 cm portion of latex.

4.3 Test Procedure

Each prepared test article section was placed on the solidified agarose surface in each of three cell culture wells. Similarly, the negative control and the positive control were each placed on the solidified agarose surface in three cell culture wells. The wells were labeled with the corresponding lab number and dosing date, and incubated at 37°C in the presence of 5% CO₂ for 24–26 hours.

Following incubation, the cultures were examined macroscopically for cell decolorization around the test article and controls to determine the zone of cell lysis (if any). After macroscopic examination, the cell monolayers were examined microscopically (100X) to verify any decolorized zones and to determine cell morphology in proximity to the articles.

All times and temperatures reported herein are approximate and are within ranges established by the external standards described in the References section of this report and/or NAMSA standard operating procedures.

5. Evaluation

Scoring for cytotoxicity was based on the following criteria:

Table 5: Test Scoring

Grade	Reactivity	Condition of Cultures
0	None	No detectable zone around or under specimen
1	Slight	Some malformed or degenerated cells under specimen
2	Mild	Zone limited to area under specimen
3	Moderate	Zone extending specimen size up to 1.0 cm
4	Severe	Zone extending farther than 1.0 cm beyond specimen

For the suitability of the system to be confirmed, the negative control must have been a grade of 0 (reactivity none) and the positive control must have produced a zone of lysis for a grade equal to or greater than a grade of 3 (reactivity moderate to severe). The test article met the requirements of the test if all three monolayers exposed to the test article showed cytotoxicity no greater than a grade of 2 (reactivity mild).

6. Results

The scores obtained were as follows:

Table 6: Individual Scores

Test/Control Articles	Zone of Lysis (mm)	Lysis Under Article	Grade	Reactivity
Test Article	(1) 0	None	0	None
	(2) 0	None	0	None
	(3) 0	None	0	None
Negative Control	(1) 0	None	0	None
	(2) 0	None	0	None
	(3) 0	None	0	None
Positive Control	(1) 7	Complete	3	Moderate
	(2) 7	Complete	3	Moderate
	(3) 7	Complete	3	Moderate

Note: (1), (2), and (3) denote replicates.

7. Conclusion

The test article showed no evidence of causing any cell lysis or toxicity. The test article met the requirements of the test since the grade was less than or equal to a grade 2 (mild reactivity).

Results and conclusions apply only to the test article tested. Any extrapolation of these data to other articles is the sponsor's responsibility.

8. Records

All raw data pertaining to this study and a copy of the final report are retained in designated NAMSA archive files in accordance with NAMSA SOPs.

9. References

International Organization for Standardization (ISO) 10993-1, Biological evaluation of medical devices - Part 1: Evaluation and testing within a risk management process (2009/Technical Corrigendum 1 2010).

International Organization for Standardization (ISO) 10993-5, Biological evaluation of medical devices - Part 5: Tests for *in vitro* cytotoxicity (2009).

International Organization for Standardization (ISO) 10993-12, Biological evaluation of medical devices - Part 12: Sample preparation and reference materials (2012).

International Organization for Standardization (ISO) 13485, Medical devices - Quality management systems - Requirements for regulatory purposes (2003/Technical Corrigendum 1 2009).

International Organization for Standardization/International Electrotechnical Commission (ISO/IEC) 17025, General requirements for the competence of testing and calibration laboratories (2005/Technical Corrigendum 1 2006).

United States Pharmacopeia 41, National Formulary 36 (USP), General Chapter <87>, Biological Reactivity Tests, In Vitro (2018).