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TEST ARTICLE: MED 200

IDENTIFICATION: N/A

P.O. NO.: VINTEKS1

DATE RECEIVED|INITIATED|COMPLETED: 10-18-2021 | 10-21-2021 | 10-27-2021

TEST PROCEDURE: Cytotoxicity Test - Direct Contact/L929 Mouse Fibroblast
Ref. Geneva Laboratories Proc. No. CC1003M
ISO Method Add. No. ISO-01B

OBJECTIVE: To assess the biological reactivity of a mammalian cell culture to component materials or finished medical products through direct contact.

CONTROL ARTICLES: Positive Control - Latex Rubber Sheet
Lot 07132020 (MSC)

Negative Control - HDPE Sheet,
Lot 02182011 (U.S. Plastics)

STABILITY: Solid test articles and the positive and negative control materials (latex rubber and HDPE sheet) are items with no known stability concerns when held at room temperature conditions. Semi-solid or liquid test articles will be stored in accordance with sponsor recommendations.

TEST SYSTEM: The test was performed using mouse fibroblast cells obtained from Diagnostic Hybrids (Cell Line L929, Lot 011509). The cells had been subcultured using a growth medium, MEM w/10% FBS. The cells were held at 37°C ±1°C/5% CO_2 during the growth phase.

The passage number/date of the subculture utilized for this test was: p767/102021-2

TEST METHODS:

A. Sample Preparation

The test article and control materials were prepared in accordance with the American National Standard ANSI/AAMI/ISO 10993-5: 2009/(R)2014.

Test Article
Dimensions: 1.027cm x 1.028cm/1.030cm x 1.042cm/1.009cm x 1.031cm

Positive Control
Dimensions: 0.990cm x 1.066cm/1.006cm x 1.017cm/1.033cm x 1.053cm

Negative Control
Dimensions: 1.029cm x 1.030cm/0.995cm x 1.052cm/1.004cm x 1.017cm

B. Cell Preparation

Prior to the exposure phase, the cells were subcultured to achieve a confluency of approximately 80 ±10% at the time of exposure. Each of the test dishes was identified with the cell line, cell passage number, and date of passage.

Just prior to exposure, each of the dishes was microscopically examined for possible contamination and to observe if the level of confluency required had been achieved.

Percentage confluency at time of use: 80-85%

Once found to meet the acceptance criteria for use in the test, individual dishes were numbered (in triplicate) to represent the controls and the test articles.

C. Cell Exposure

On the day of testing, the subculture medium was carefully removed from each test dish and replaced with a fresh 0.8 mL aliquot of culture medium containing an antibiotic/antimycotic solution:

Medium Used: MEM w/5% FBS Lot No. 10112021

A single test article or control specimen was placed gently in the center of each dish, so as not to disrupt the cell monolayer. Triplicate cultures were prepared for each test article and positive and negative controls.

The test dishes, along with three (3) dishes with medium only (Medium Negative Controls), were then placed in the 37°C ±1°C/5% CO_2 incubator to initiate the exposure interval.

Exposure Date: 10-21-2021
Exposure Interval: 24 Hours

D. Cellular Staining and Fixation

After completion of the incubation period, the cells were stained with 0.01% Neutral Red Solution to facilitate response grading. The test article and control materials were removed from the dishes at this time.

The stained cells were then fixed by the addition of 10% buffered formalin.

E. Observations

A preliminary microscopic examination of the cells was made prior to staining and before the control and test specimens were removed from the cell monolayer.

Following the staining process, the cellular responses were then evaluated microscopically and macroscopically (by examining the dishes against a white surface) and the results recorded.

E. Observations (Cont'd)

See table below for grading guidelines.

Grade(1)	Reactivity	Description of Reactivity Zone(2)
0	None	No detectable zone around or under specimen
1	Slight (3)	Some malformed or degenerated cells under specimen(s)
2	Mild	Zone limited to area under specimen
3	Moderate	Zone extends 0.45 to 1.0 cm beyond specimen
4	Severe	Zone extends greater than 1.0 cm beyond specimen

NOTE(1): The use of the above Grading Table is contingent on the test article meeting the minimum surface area requirements of ≥100 mm^2. Should samples of smaller dimensions be tested, the reactivity (if any) would be expected to be less and the grading would need to be justified.

NOTE(2): The extent of the Reactivity Zone is the maximum measured distance from the edge of the specimen to the margin of monolayer where degenerated cells are no longer observed. Where described as "under specimen", this maximum measured distance is limited to <0.45 cm beyond the specimen.

NOTE(3): To be interpreted as "slight" reactivity, no more than 50% of the cells under the specimen may exhibit reactivity as rounding and/or lysis.

F. Additional Methods:

Any additional steps required to complete the test that were not described above are indicated here:

Samples were presaturated with MEM and tested with coated side contacting the cells.

RESULTS: See table below for the cellular responses from the controls and test article.

		Macroscopic Reading (Zone Dimensions)	Microscopic Reading (% Rounded/Lysed)	Grade
Monolayer Negative Control	1	No detectable zone	0% / 0%	0
	2	No detectable zone	0% / 0%	0
	3	No detectable zone	0% / 0%	0
Material Positive Control	1	Zone 3.4 x 3.4cm Greatest distance from specimen 1.382cm	100% / 100% (in zone)	4
	2	Zone 3.4 x 3.4cm Greatest distance from specimen 1.264cm	100% / 100% (in zone)	4
	3	Zone 3.4 x 3.4cm Greatest distance from specimen 1.264cm	100% / 100% (in zone)	4
Material Negative Control	1	No detectable zone	0% / 0%	0
	2	No detectable zone	0% / 0%	0
	3	No detectable zone	0% / 0%	0
Test Article	1	Zone 1.205 x 1.285cm Greatest distance from specimen 0.205cm	100% / 90-95% (in zone)	2
	2	Zone 1.223 x 1.242cm Greatest distance from specimen 0.260cm	100% / 90-95% (in zone)	2
	3	Zone 1.265 x 1.265cm Greatest distance from specimen 0.169cm	100% / 90-95% (in zone)	2

Date Read: 10-27-2021
Read By: Daryl Meyer

OBSERVATIONS:

N/A

INTERPRETATION OF RESULTS:

A. Assay controls must meet the acceptance criteria for a valid assay.

All negative control responses must be no greater than Grade 0 unless there was documented mechanical stress, in which case a Grade 1 (Slight) response is acceptable.

Positive control responses must be no less than Grade 3.

B. The responses observed from the test article are interpreted according to current USP guidelines.

Grades of 0 (None), 1 (Slight), or 2 (Mild) indicate that the test article meets assay acceptance requirements.

Grades of 3 (Moderate) or 4 (Severe) indicate that the test article does not meet assay acceptance requirements.

C. No statistical analysis of the data was made.

CONCLUSION:

The Grade 2 response to the sample preparation is considered to be non-cytotoxic (i.e., meets ISO test acceptance requirements of no more than Grade 2 reactivity).

ANALYST: Daryl A. Meyer DATE: 10-28-2021

ACCEPTED BY: _____ DATE: 10-28-2021
Technical Reviewer

Q.A. SIGNATURE: Elaine Maloney DATE: 10-29-21