No.L1538

Test Report

TEST ITEMS

Test for *in vitro* cytotoxicity (Agar diffusion test)

TEST ARTICLE

Insertion Tube & Universal tube for Flexible endoscope
(spec: BF-GIF-CF-LG)

IDENTIFICATION №

060506

SPONSOR

Shanghai Yanshun Scope Parts & Accessories Co., Ltd.

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SUMMARY

An *in vitro* cytotoxicity study was conducted to assess the potential for cytotoxicity of the test article: **Insertion Tube & Universal tube for Flexible endoscope (spec: BF-GIF-CF-LG)**, based on the International Organization for Standardization ISO 10993-5-1999: Biological Evaluation of Medical Devices - Part 5: Tests for *in vitro* Cytotoxicity.

The test article, negative control and positive control samples were prepared in appropriate size. Each sample was placed in contacted with the surface of an agar layer overlaying a monolayer of L-929 Mouse Fibroblast cells which are stained with neutral red vital stain at the bottom of the plate. After incubation, the cells were examined to access the size of the decolourized zone and the extent of the cell lysis. Cytotoxic effects were determined by qualitative means, and the grade of cytotoxic reactivity was evaluated on 0~3.

Under the conditions of this study, the test article showed a slight cytotoxicity. The negative control and the positive controls performed as anticipated.

Study and Supervisory
Personnel: MENG Aiying
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Study Director: Sun Jiao
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July 4, 2006
Date Completed

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INTRODUCTION

The test was performed in order to determine the test article's potential for cytotoxicity. The test article was received on May.16, 2006. The cells were seeded on May.22, 2006, and the final observations were concluded on May.24, 2006.

This study was completed in the Lab of Shanghai Biomaterials Research & Test Center (SBRTC). SBRTC was conducted in accordance with the provisions of the ISO/IEC 17025-2005.

MATERIALS

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

Test Article:	Insertion Tube & Universal tube for Flexible endoscope (spec: BF-GIF-CF-LG)
Identification №:	060506
Storage Conditions:	Room temperature
Cell culture medium:	GIBCO's Minimum Essential Medium, supplemented with 10% calf serum and 1% L-glutamine.
Test Article Preparation:	The black outer cover of the test article was cut to a round sheet in 5mm diameter for testing.
Negative Control Preparation:	Current SBRTC negative control, high-density polyethylene sheet, as the same size as the test article.
Positive Control:	Current SBRTC positive control, an organo-tin stabilized polyvinylchloride sheet, as the same size as the test article.
Sample Disposition:	Any remaining sample was discarded

METHODS

Test System Management:

Mouse fibroblast cells (L 929, from the cell bank of CAS), were propagated at 37℃ in sealed flasks containing GIBCO's MEM, supplemented with 10% calf and 1% L-glutamine. For this study, two Φ 90mm plates was seeded 10ml suspension of 3.0×10^5 cells per milliliter, and incubated at 37℃ for 24h in order to obtain a confluent monolayer of cells.

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Preparation of Agar Overlay:

Equal amounts of double strength Minimum Essential Medium (2x MEM) and 3% agar were combined to form an MEM-agar mixture. 10ml of the MEM-agar mixture was then placed in the cell culture plates that had a confluent monolayer of cells, and allowed to solidify over the cells to form the agar overlay. 10ml freshly prepared neutral red vital stain was added gently to cover the entire solidified agar surface. The strong light was shield from for 15min.

Experimental Procedure:

Two replicate test articles, one negative control and one positive control were applied symmetrically to the surface of the agar of each of two plates with the edge of the samples approximately 15mm from the edge of the plate. After the application of the samples, the plates were placed in a 37℃ incubator, under 5% carbon dioxide in air and incubated for 24h.

Following incubation, the response of the vitally stained monolayer was evaluated with respect to the size of the decolourized zone and the extent of the cell lysis under and around the sample.

Scoring for cytotoxicity was bases on the following criteria:

Zone index (Z)	Description of zone	Lysis index (L)	Description of zone
0	No detectable zone around or under sample	0	No observable lysis
1	Zone limited to area sample	1	Up to 20% of zone lysed
2	Zone not greater than 5mm in extension from sample	2	Up to 20% to 40% of zone lysed
3	Zone not greater than 10mm in extension from sample	3	Up to 40% to 60% of zone lysed
4	Zone greater than 10mm in extension from sample, but not involving entire dish	4	Up to 60% to 80% of zone lysed
5	Zone involving entire dish	5	Over 80% lysed within the zone

Cytotoxicity scale	Response Index (R/Z)	Interpretation
0	0/0	Noncytotoxic
1	1/1	Mildly cytotoxic
2	2/2~3/3	Moderately cytotoxic
3	4/4~5/5	Severely cytotoxic

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RESULTS

The Response Index (R/Z) of test article was 1/0.
Results and conclusions apply only to the test article tested. No further evaluation of these results is made by Shanghai Biomaterials Research & Test Center.

CONCLUSION

Under the condition of this study, the test article showed a slight cytotoxicity. The negative control and the positive control performed as anticipated.

RECORD STORAGE

All raw data pertaining to this study and a copy of the final report are to be stored in the designated archive files at Shanghai Biomaterials Research & Test Center.

PHOTOGRAPH OF THE TEST ARTICLE

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