TESTING
CNAS L1538

Test Report

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TEST ITEMS

Test for *in vitro* cytotoxicity (MTT cytotoxicity test)

TEST ARTICLE

latex surgical gloves
<LOT: 131025; Size: 7.5>

IDENTIFICATION №

140690

MANUFACTURER

Tianchang Anrui Medical Equipments Co., Ltd.
<Address: Renhe Industrial District Tianchang City Anhui Province>

SPONSOR

TÜV SÜD Certification and Testing (China) Co., Ltd. Shanghai Branch
<Address: № 88 Hengtong Rd>

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201, Building 2, № 427 Jumen Road, Shanghai, China Zip Code: 200023
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SUMMARY

An in vitro cytotoxicity study was conducted to assess the potential for cytotoxicity of the test article, latex surgical gloves , based on the International Organization for Standardization ISO 10993-5:2009: Biological evaluation of medical devices - Part 5: Tests for in vitro cytotoxicity; ISO 10993-12:2012: Biological evaluation of medical devices - Part 12: Sample preparation and reference materials.

Four concentrations (100%, 75%, 50%, and 25%) of the test article extracts, the blank, 100% of the negative control and the positive control were prepared using Minimum Essential Medium (MEM) supplemented with 10% fetal bovine serum. The semi-confluent monolayers of L-929 mouse fibroblast cells were incubated with the test extract, the blank and other two controls, supplemented with 10% fetal bovine serum in a 96-well microplate respectively at 37℃ under the condition of 5% CO_2. At 24h, the MTT colorimetric assay was employed and the plate was read on a microplate reader at 570 and 650 nm. The viability of the cells was calculated.

Under the condition of this study, the viability of 100% extract of the test article was 4 %. It can be considered that the test article extracts had a cytotoxic potential.

Study and Supervisory Personnel: XU Yuan
HUANG Zhewei

Study Director: Sun Jiao Oct 8. 2014

SUN Jiao, Ph.D. Date Completed

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INTRODUCTION

The study was performed in order to determine whether leachables extracted from the test article would cause cytotoxicity. This test was conducted based on the requirements of ISO 10993-5:2009: Biological evaluation of medical devices - Part 5: Tests for *in vitro* cytotoxicity. The test article was received on Jun. 27, 2014. The cells were first exposed to the extract on Jul. 22, 2014, and the final observations were concluded on Jul. 23, 2014.

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:	latex surgical gloves <LOT: 131025; Size: 7.5>
Identification №:	140690
Sterilization Status:	Sterilized
Temperature Tolerance:	37℃(declaration from the sponsor)
Storage Conditions:	Room temperature
Extraction Vehicle:	GIBCO's Minimum Essential Medium supplemented with 10% fetal bovine serum and 1% L-glutamine
Test Extract Preparation:	Based on the ISO ratio of 6cm^2: 1ml [Surface area of the test article to volume of extraction vehicle], 100cm^2 of the test article were covered 16.7ml of extraction vehicle for preparing the test extract at 37℃ for 24 hours. The extract was used immediately after extraction.
Blank Preparation:	The extraction vehicle not containing the test sample, retained in a vessel identical to that which holds the test article and subjected to conditions identical to those to which the test sample is subjected during its extraction.
Negative Control Preparation :	Current SBRTC negative control, the ratio of 3cm^2 high-density polyethylene: 1ml [surface area of the test article to volume of extraction vehicle] was used and extracted at 37℃ for 24 hours.
Positive Control Preparation:	Current SBRTC positive control, the ratio of 6cm^2 organo-tin stabilized polyvinylchloride sheet : 1ml [surface area of the test article to volume of extraction vehicle] was used and extracted at 37 ℃ for 24 hours.
Condition of Extracts:	All the extracts of the test and controls were clear and without any special treatments.

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METHODS

Test System Management:

Mouse fibroblast cells (L 929, from the cell bank of Shanghai Institutes for Biological Sciences), were cultured in MEM supplemented with 10% fetal bovine serum and 1% L-glutamine at 37℃ in a gaseous environment of 5% carbon dioxide (CO_2). A 96-well microplate method was employed for the MTT colorimetric assay. Each well was seeded 100μl suspension of 1×10^4 cells, and incubated at 37℃ in 5% CO_2 atmosphere for 24h prior to use.

Experimental Procedure:

After incubation, the growth medium was replaced with 100μl four concentrations (100%, 75%, 50% and 25%) of the test extract, 100% of the negative control and the positive control, the blank (row 2 and 11) respectively. Six replicates were prepared for each group. The 96-well plate were incubated at 37℃ in 5% CO_2 for 24h.

After 24 h treatment, the culture medium was removed carefully from the plates. 50μl of the MTT (Sigma, 1mg/mL) solution was then added to each test well and the plates were further incubated for 2 h at 37℃ in a 5% CO_2 atmosphere. Then the MTT solution was removed and 100μl isopropanol per well was added and shake for 10min by gently. The plate was read on a microplate reader at 570 nm (reference wavelength 650 nm). The viability of the cells was calculated according to the formula below:

$$\text{Viab. \%} = \frac{100 \times OD_{570e}}{OD_{570b}}$$

Where

OD_{570e} is the mean value of the measured optical density of the extracts of the test sample;

OD_{570b} is the mean value of the measured optical density of the blanks;

A test meets acceptance criteria if the left and the right mean of the blanks do not differ by more than 15% from the mean of all blanks. If the viability of the test sample was reduced to <70% of the blank, it had a cytotoxic potential. The 50% extract of the test sample should have at least the same or a higher viability than the 100% extract; otherwise the test should be repeated.

RESULTS

Group	The optical density (570nm−650 nm)	Viab. %
100% of the negative control	0.776±0.031	98
100% of the test extract	0.035±0.006	4
75% of the test extract	0.084±0.006	11
50% of the test extract	0.133±0.011	17
25% of the test extract	0.171±0.016	22
100% of the positive control	0.028±0.005	4
The blank (row 2)	0.790±0.043	/
The blank (row 11)	0.791±0.039	/

Note: n=6

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The mean value of optical density of the blank was 0.790±0.039. Both the left (row 2) and the right (row 11) mean of the blanks were less than 15% from the mean of all blanks.

Results and conclusions apply only to the test article tested. No further evaluation of these results was made by Shanghai Biomaterials Research & Test Center.

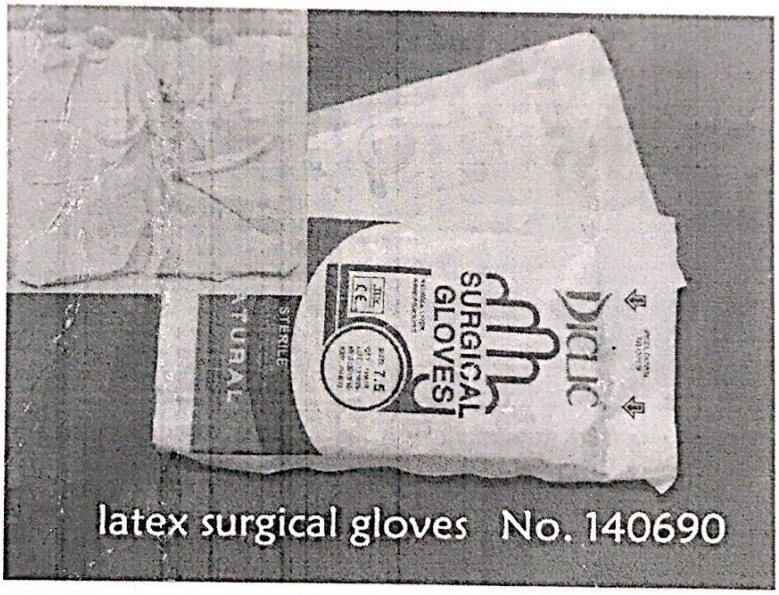
CONCLUSION

Under the condition of this study, the viability of 100% extract of the test article was 4 %. It can be considered that the test article extracts had a cytotoxic potential.

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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