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Test Report

Report Number: SSMT-R-2020-03009-01B

Sample Name: menstrual cup

Study Title: In Vitro Cytotoxicity Test

Standard: ISO 10993-5:2009

GB/T 16886.5-2017

Test facility

Jiangsu Science Standard Medical Testing Co., Ltd.

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Sponsor

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Explanation

1. Please apply for rechecking within 15 days of receiving the report if there is any objection.
2. Any erasure or without special testing seal renders the report null and void.
3. The report is only valid when signed by the persons who edited, checked and approved it.
4. The result relate only to the articles tested.
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Conclusion

The study was to investigate the potential cytotoxicity of the test sample. The extract of the test article was added to L-929 cells and then incubated at 37℃ in 5% CO_2 for 24 hours. After the incubation, observe the cell morphology. The results were detected with MTT method. The results showed that the cytotoxicity ratio of the 100 % test article extract was 85.9% and the results of control groups showed the test was valid.

Under the conditions of this study, the extract of the test article did not show potential toxicity to L-929 cells.

Study verification and signature

The study was carried out in accordance with the standard operating procedure. The test process was conducted in compliance with the requirements of CNAS-CL01:2018 (IDT ISO/IEC 17025:2017) and RB/T 214-2017.

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Checked by Bella Pi — 2020.10.26 (Date)

Approved by Daisy Zhang (Authorized signatory) — 2020.10.28 (Date)

Jiangsu Science Standard Medical Testing Co., Ltd.

1.0 Purpose

The purpose of the test is to determine the potential cytotoxicity toxicity of a mammalian cell culture (mouse fibroblast L-929 cells) in response to the test article.

2.0 Standard

Biological evaluation of medical devices Part 5: Tests for In Vitro Cytotoxicity (ISO 10993-5:2009)

Biological evaluation of medical devices Part 12: Sample preparation and reference materials (ISO 10993-12:2012)

Biological evaluation of medical devices Part 5: Tests for In Vitro Cytotoxicity (GB/T 16886.5-2017)

Biological evaluation of medical devices Part 12: Sample preparation and reference materials (GB/T 16886.12-2017)

3.0 Test and control articles

3.1 Test article (The information about the test article was supplied by the sponsor wherever applicable.)

Test article name: menstrual cup

Sterilization state: Not sterilized

Model/Size: N/S

Lot/ Batch#: N/S

Physical State: Solid

Color: See the photo

Density: N/S

Stability: N/S

Solubility: N/S

Test Article Material: silica gel

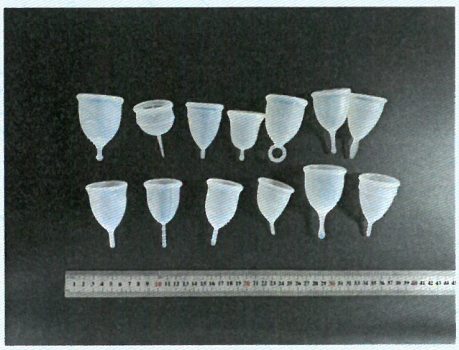
Packing Material: N/S

Storage Condition: Room temperature

Manufacturers: Xi'an Furuize Biotechnology Co., Ltd.

Manufacturer address: Room 11131 , Building 3,i Duhui, No.11 ,Tangyan South Road ,Zhangba Street Office ,High-tech Zone ,Xi'an City, Shaanxi, China.

Sample photograph:



3.2 Control Articles

3.2.1 Negative Control Article Name: High Density Polyethylene

Manufacturer: Jiangsu haiaosihui biotechnology co., LTD.

Size: 1.6 mm thick, 300*300 mm

Lot/ Batch#: M02F017

Physical State: Solid

Color: White

Storage Conditions: Room temperature

3.2.2 Positive Control Article Name: ZDEC

Manufacturer: Tokyo Into Industrial Co., Ltd.

Size: 25 g

Lot/ Batch#: DUDQG-JF

Physical State: Solid

Color: White

Storage Condition: Room temperature

Concentration: 0.1%

3.2.3 Blank Control Name: MEM medium, with addition 10% FBS

Physical State: Liquid

Color: Pink

Storage Condition: 4 ℃

4.0 Identification of test system

Mouse fibroblast L-929 cells obtained from ATCC CCL1 (NCTC clone 929).

5.0 Justification of test system

5.1 Historically, mouse fibroblast L-929 cells have been used for cytotoxicity studies because they demonstrate sensitivity to extractable cytotoxic articles.

5.2 The test article was extracted and administered in vitro to mouse fibroblast L-929 cells through a solvent compatible with the test system. This was the optimal route of administration available in this test system as recommended in the standard.

6.0 Instruments and Reagents

6.1Instruments

Vertical pressure steam sterilizer (SSMT-281)

CO_2 Incubator (SSMT-279)

Biological microscope（SSMT-278）

Clean bench (SSMT-028)

Bench type low speed centrifuge (SSMT-048)

Vapour-bathing Constant Temperature Vibrator (SSMT-004)

Electronic Balance (SSMT-015)

Multiskan Spectrum Microplate Spectrophotometer (SSMT-139)

Mini Vibrator (SSMT-311)

6.2 Reagents

FBS

MEM

Trypsin

Penicillin, Streptomycin sulfate

PBS

MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyletrazolium bromide)

Isopropyl alcohol

7.0 Experiment design and dose

7.1 Sample preparation

Aseptic extracting the test article (test article to volume of vehicle) according to the table below. Sealed and incubated in Vapour-bathing Constant Temperature Vibrator at 37 ℃ and 60 rpm for 24 hours. After the extraction, check the extraction changes, and immediately use for the experiment, the leach was not filtered, centrifuged or diluted. No pH adjustment.

Table 1 Sample preparation

Sampling		Aseptic Agitation Extraction In Inert Container				Final Extract
Sampling Manner	Actually sampling	Extraction solvent	Extraction ratio	Solvent volume	Condition	Clear or Not
Take the whole	12.08 g	MEM medium (10% FBS)	0.2 g : 1 ml	60.4 ml	37 ℃, 24 h	Clear

The blank control (MEM medium, with addition 10% FBS) and negative/positive controls were prepared in the same condition.

7.2 Test method

Aseptic procedures were used for handling cell cultures.

L-929 cells were cultured in MEM medium (10% FBS, Penicillin 100 U/ml, Streptomycin sulfate 100 μg/ml) at 37℃ in a humidified atmosphere of 5% CO_2, then digested by 0.25% trypsin containing EDTA to get single cell suspension. And obtain a 1×10^5 cells/ml suspension by centrifuging (200 g, 3 min) and re-dispersing in MEM medium finally.

The suspended cells were dispensed at 100 μl per well in 96-well plate, and cultured in cell incubator (5% CO_2, 37℃, >90%humidity). Cell morphology was evaluated to verify that the monolayer was satisfactory.

After the cells grew to form a monolayer, original culture medium was discarded. The 96-well plates were then treated with 100 μl of extract of test article (100%, 75%, 50%, 25%), control article, negative article (100%) and positive article (100%) respectively. The 96-well plate was incubated at 37℃ in cell incubator of 5% CO_2 for 24 h. Six replicates of each test were tested.

After 24 h incubation, observe the cell morphology first and then discard the culture medium. A 50 μl aliquot of MTT (1 mg/ml) was added to each well and then incubated at 37℃ in a humidified atmosphere of 5% CO_2 for 2

hours. The liquid in each well was tipped out and 100 μl isopropanol was added to each well to suspend the cell layer. The microporous plate was vibrated for 10 min and monitored by the optical density at 570 nm on the microplate analyzer.

7.3 Statistical method

Mean±standard deviation ($\bar{x}$ ±s)

Viab. %=100×OD_{570e}/OD_{570b}

Where: OD_{570e}——is the mean value of the measured optical density of test sample/negative control/positive control;

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

7.4 Observation of the cell morphology

Table 2 Observation of the cell morphology

Grade	Conditions of all cultures
0	Discrete intracytoplasmatic granules, no cell lysis, no reduction of cell growth.
1	Not more than 20 % of the cells are round, loosely attached and without intracytoplasmatic granules, or show changes in morphology; occasional lysed cells are present; only slight growth inhibition observable.
2	Not more than 50 % of the cells are round, devoid of intracytoplasmatic granules, no extensive cell lysis; not more than 50 % growth inhibition observable.
3	Not more than 70 % of the cell layers contain rounded cells or are lysed; cell layers not completly destroyed, but more than 50 % growth inhibition observable.
4	Nearly complete or complete destruction of the cell layers.

8.0 Evaluation criteria

8.1 The 50% extract of the test article should have at least the same or a higher viability than the 100% extract. Otherwise the test should be repeated.

8.2 The lower the Viab.% value, the higher the cytotoxic potential of the test article is.

8.3 If viability is reduced to < 70% of the blank, it has a cytotoxic potential.

8.4 The Viab.% of the 100% extract of the test article is the final result.

9.0 Results of the test

Table 3 Results of the cell vitality

Group	$\bar{x}$ ±s	Viability%	The morphology of the extracted cells was observed under the microscope
Blank control	0.875±0.090	100.0	0

Negative control	0.878±0.043	100.3	0
Positive control	0.074±0.012	8.5	4
100% test article extract	0.752±0.035	85.9	0
75% test article extract	0.858±0.033	98.0	0
50% test article extract	0.839±0.012	95.9	0
25% test article extract	0.874±0.043	99.9	0
Quality check	The mean OD_{570} of blanks is ≥ 0.2. The left (row2) and the right (row11) mean of the blanks do not differ by more than 15 %. The test meets the acceptance criteria.		
Conclusion	Under the conditions of this study, the test article did not show potential toxicity to L-929 cells.		

10.0 Deviation statement

There was no deviation from the approved standard operating procedure which were judged to have any impact on the validity of the data.

11.0 Record

All the original data and records related to this test and copies of the final report are retained in the archives of Science Standard Medical Testing.

12.0 Confidentiality agreement

Statements of confidentiality were as agreed upon prior to study initiation.