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CNAS L2954

Final Report

Report Number: SDWH-M202007193-1(E)

In Vitro Cytotoxicity Test of 5040TPU Low noise medical cable

According to ISO 10993-5: 2009
MTT Method
MEM with 10%FBS extract

Sponsor: Shenzhen YONGQIANGFU Industrial CO.,Ltd

Address: 2Bldg,No.2 Industrial park,Xinwei Village ,Dalang LongHua Town,Shenzhen City

检验检测专用章 (2)

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Content

Supplementary Explanation

(1) Please apply for rechecking within 15 days of receiving the report if there are any objections.

(2) Any erasure or without special inspection and 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 results relate only to the articles tested.

(5) The report shall not be reproduced except in full without the written approval of the institute.

Quality Assurance Statement

The Quality Assurance Unit inspected/audited this study in compliance with the following GLP regulations:

Good Laboratory Practice (GLP) Regulation 21 CFR Part 58, U.S. Food and Drug Administration (FDA).The laboratory is exempt from the following provisions: 21 CFR Part 58.105 Test and Control Article Characterization, and Part 58.113 Mixtures of Articles with Carriers.

The Quality Assurance Unit conducted inspections on the following dates. The findings were reported to the Study Director and to the Testing Facility Management.

The final report was reviewed by the Quality Assurance Unit. The final report accurately describes the test methods in accordance with standard operating procedures, and the results are consistent with raw data of non-clinical studies conducted according to the study protocol.

Inspections	Date of Inspection	Date Reported to Study Director	Date Reported to Testing Facility Management.
Study Protocol	2020-12-25	2020-12-25	2021-01-07
Study Procedure	2020-12-29	2020-12-29	2021-01-07
Raw Data	2021-01-07	2021-01-07	2021-01-07
Final Report	2021-01-07	2021-01-07	2021-01-07

Quality Assurance Unit: Ou Tingting 2021-01-07

Quality Assurance Date

GLP Compliance Statement

This study was fully in accordance with the technical requirements of the study protocol.

This study was conducted in compliance with Good Laboratory Practice (GLP) Regulation 21 CFR Part 58, U.S. Food and Drug Administration (FDA).

The laboratory is exempt from the following provisions: 21 CFR Part 58.105 Test and Control Article Characterization, and Part 58.113 Mixtures of Articles with Carriers.

Verification Dates

Test Article Receipt	2020-12-24
Protocol Effective Date	2020-12-25
Technical Initiation Date	2020-12-25
Technical Completion Date	2020-12-30
Final Report Completion Date	2021-01-08

Edited by: Chenrongrong 2021-01-06

Date

Reviewed by: Zhu Yuting 2021-01-08

Study Director Date

Approved by: Wang Yijie 2021-01-08

Authorized Signatory Date

Sanitation & Environment Technology Institute, Soochow University

Summary

1 Test Article

Test Article Name	5040TPU Low noise medical cable
Manufacturer	Shenzhen YONGQIANGFU Industrial CO.,Ltd
Address	2Bldg,No.2 Industrial park,Xinwei Village ,Dalang LongHua Town,Shenzhen City
Model	5040TPU
Lot/Batch	N/A

2 Main Reference

ISO 10993-5: 2009 Biological evaluation of Medical Devices—Part 5: Tests for *in vitro* Cytotoxicity

3 Test Method

Potential toxicity of test article was evaluated using MTT in accordance with ISO 10993-5: 2009 Biological evaluation of Medical Devices—Part 5: Tests for *in vitro* Cytotoxicity.
Study protocol number: SDWH-PROTOCOL-GLP-M202007193-1.

4 Conclusion

Under the conditions of this study, the test article extract did not show potential toxicity to L929 cells.

Test Report

1 Purpose

The purpose of the test is to determine the biological reactivity of a mammalian cell culture (mouse fibroblast L929 cells) in response to the test article.

2 Reference

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.

3 Compliance

Good Laboratory Practice Regulations, 21 CFR, Part 58.
ISO/IEC 17025:2017 General requirements for the competence of testing and calibration laboratories (CNAS—CL01 Accreditation criteria for the competence of testing and calibration laboratories) China National Accreditation Service for Conformity Assessment LABORATORY ACCREDITATION CERTIFICATE Registration No. CNAS L2954.
RB/T 214—2017 Competence assessment for inspection body and laboratory mandatory approval—General requirements for inspection body and laboratory Certification and Accreditation Administration of the People's Republic of China INSPECTION BODY AND LABORATORY MANDATORY APPROVAL Certificate No. CMA 180015144061.

4 Identification of Test and Control Articles

4.1 Test Article

Test Article Name	5040TPU Low noise medical cable
Manufacturer	Shenzhen YONGQIANGFU Industrial CO.,Ltd
Address	2Bldg,No.2 Industrial park,Xinwei Village ,Dalang LongHua Town,Shenzhen City
Test Article Initial State	Non-sterile
CAS Number	N/A
Model	5040TPU
Size	N/A
Lot/Batch	N/A
Raw Material	NA
Packaging Material	N/A
Physical State	Solid
Color	Green
Density	N/A
Stability	NA
Solubility	N/A
Storage Condition	Room temperature
Intended Use	N/A
Additional Information	N/A

The information about the test article was supplied by the sponsor wherever applicable.
The Sponsor is responsible for all test article characterization data as specified in the GLP regulations.

4.2 Control Article

4.2.1 Negative Control

Negative Control Article Name: High Density Polyethylene

Manufacturer: U.S. Pharmacopeial Convention (USP)

Size: 3 Strips

Lot/ Batch#: K0M357

Physical State: Solid

Color: White

Stability: Stable at room temperature

Storage Conditions: Room temperature

Extraction vehicle: MEM medium, with addition 10% FBS

4.2.2 Positive Control

Positive Control Article Name: Zinc diethyldithiocarbamate

Manufacturer: Sigma

Size: 25g

Lot/ Batch#: MKCB2943V

Concentration: 1%

Solvent: MEM medium, with addition 10% FBS

Physical State: Powder

Color: White

4.2.3Blank Control

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

Physical State: Liquid

Color: Pink

Storage Condition: 4 ±2℃

5 Equipment and Reagents

5.1 Equipment

Equipment Name	Equipment Number	Calibration Expire
Autoclave	SDWH2204	2021-03-25
Constant temperature vibrator	SDWH2109	2021-09-02
Steel straight scale	SDWH463	2021-07-06
Electronic Balance	SDWH2601	2021-05-21
Electronic Balance	SDWH230	2021-04-25
CO_2 Incubator	SDWH021	2021-03-25
Inverted microscope	SDWH037	2021-04-25
Clean bench	SDWH454	2021-04-26
Power Wave Microplate Reader	SDWH2386	2021-05-17

5.2 Reagents

Reagent Name	Manufacturer	LOT
(3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyletrazolium bromide)	SIGMA	MKCK3153
FBS	CORNING	35081002

MEM	HyClone	AF29549370
Trypsin	GiBco	2085461
Penicillin, Streptomycin sulfate	GiBco	2211091
PBS	GiBco	8120015
99.9%Isopropanol	Sinopharm Chemical Reagent Co., Ltd	20200313

6 Identification of Test System

L929 mouse fibroblast cells obtained from ATCC (American Type Culture Collection), USA.

7 Justification of Test System and Route of Administration

Historically, mouse fibroblast L929 cells have been used for cytotoxicity studies because they demonstrate sensitivity to extractable cytotoxic articles.

The test article was extracted and administered in vitro to mouse fibroblast L929 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 guidelines.

8 Experimental Design

8.1 Preparation of Extracts

8.1.1 Pretreatment

8.1.1.1 Test samples

Wipe the entire surface of test sample for 3 times using a piece of soft cloth dampened with Ethanol (75%), then rinse the sample with sterilized water for 2 min. Leave the sample to air dry in clean bench.

8.1.1.2 Control samples

Same as the test sample.

8.1.2 Extraction

Under aseptic conditions, samples were taken according to the sampling method (Random sampling). Extractions shall be performed with agitation in closed inert containers according to the extraction ratio listed in the following table (sample: extraction vehicle). The extraction vehicle is MEM medium containing 10% fetal bovine serum. After the extraction was completed, record the condition of the extracts and any changes in the extraction solvent (pre- and post-extraction). The extracts will be used immediately for test.

Samples	Actual Sampling	Extract Procedure			Final Extract
		Extract Ratio	Volume of Extraction Vehicle	Condition	
Test	30 cm^2	3 cm^2 : 1 mL	10.0 mL	37 ℃, 24 h	Clear
Negative Control	30 cm^2	3 cm^2 : 1 mL	10.0 mL	37 ℃, 24 h	Clear
Blank Control	/	/	10.0 mL	37 ℃, 24 h	Clear
Positive Control	0.5 g	1.0 g:100 mL	50.0 mL	37 ℃, 24 h	Not Clear

There was no change in the extraction solvent for the test samples (pre- and post-extraction). The final extract of the test samples was not subjected to processes such as pH adjustment, filtration, centrifugation, or dilution. Only the positive control extract was filtered before use since the

powder of the positive sample suspended in the extraction solvent can adversely affect the test system.

8.2 Experimental Procedure

Aseptic procedures were used for handling cell cultures.

L929 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 100μL per well in 96-well plate, and culture it in cell incubator (5% CO_2,37℃,>90%humidity) for 24h. 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 100μL of extract of test article (100%、75%、50%、25%), control article, negative article (100%) and positive article (100%) respectively. Incubate the 96-well plate at 37℃ in cell incubator of 5% CO_2 for 24 h. Five replicates of each test were tested.

After 24 h incubation, observe the cell morphology first and then discard the culture medium. A 50μL 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 h. The liquid in each well was tipped out and 100 μL 99.9% isopropanol was added to each well to suspend the cell layer.

Evaluate the suspension above with a dual-wavelength spectrophotometer with the measurement wavelength at 570 nm and reference wavelength at 650 nm.

8.3 Results

The cell viability of 100% test article extract was 101.6%. See Annex 1, table 1 and table 2 for specific results.

8.4 Quality Check

No cytotoxic effect is observed for the negative controls and a cytotoxic effect is elicited by the positive controls.

The absolute value of optical density, OD_{570}, obtained in the untreated blank indicates the 1×10^4 cells seeded per well have grown exponentially with normal doubling time during the two days of the assay.

The mean OD_{570} of blanks is not less than 0.2.

Check for systematic cell seeding errors, blanks are placed both at the left side (row 2) and the right side (row 11) of the 96-well plate (row 1 and row 12 shall not be used). The left and the right mean of the blanks do not differ by more than 15 % from the mean of all blanks.

8.5 Statistical Method

SPSS16.0 will be used to calculate the Mean ±SD of each group.

$$\text{Viab.}(\%) = 100 \times \overline{(OD_{570}-OD_{650})}_{\text{Sample}} \Big/ \overline{(OD_{570}-OD_{650})}_{\text{Blank}}$$

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

8.6 Evaluation Criteria

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

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

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

9 Conclusion

Under the conditions of this study, the test article extract did not show potential toxicity to L929 cells.

10 Record Storage

All raw data pertaining to this study and a copy of the final report are to be retained in designated SDWH archive.

11 Confidentiality Agreement

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

12 Deviation Statement

There were no deviations from the approved study protocol which were judged to have any impact on the validity of the data.

Annex 1 Results

Table 1 Observation of the Cell morphology

Group	After inoculation	Before treated with extract	24 h after treatment
Blank control	Discrete intracytoplasmatic granules, no cell lysis, no reduction of cell growth.	Discrete intracytoplasmatic granules, no cell lysis, no reduction of cell growth.	Discrete intracytoplasmatic granules, no cell lysis, no reduction of cell growth.
Negative control			Discrete intracytoplasmatic granules, no cell lysis, no reduction of cell growth.
Positive control			Nearly complete or complete destruction of the cell layers.
100% Test article extract			Discrete intracytoplasmatic granules, no cell lysis, no reduction of cell growth.
75%Test article extract			Discrete intracytoplasmatic granules, no cell lysis, no reduction of cell growth.
50% Test article extract			Discrete intracytoplasmatic granules, no cell lysis, no reduction of cell growth.
25% Test article extract			Discrete intracytoplasmatic granules, no cell lysis, no reduction of cell growth.

Table2 Results of the Cell Vitality

Group	Value of OD Mean±SD	Cell Vitality %
Blank control	0.7437±0.075	100.0
Negative control	0.6958±0.019	93.6
Positive control	0.2532±0.021	34.0
100% Test article extract	0.7558±0.067	101.6
75%Test article extract	0.7396±0.026	99.4
50% Test article extract	0.7562±0.028	101.7
25% Test article extract	0.7428±0.048	99.9

Annex 2 Photograph of Test Article

Annex 3 Information Provided by Sponsor

1 Production Process

Not supplied by sponsor.

2 Other Information

Not supplied by sponsor.

End of Report