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CNAS L2954

Final Report

Report Number: SDWH-M202003265-1(E)

In Vitro Cytotoxicity Test of Disposable Nitrile Examination Glove

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

Sponsor: Shanxi Hongjin Plastic Technology Co., Ltd

Address: Coal Bed Gas Industrial Zone, Qu'e Town, Daning County, Linfen City, Shanxi Province

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	2020-06-30	2020-06-30	2020-07-28
Study Procedure	2020-07-09	2020-07-09	2020-07-28
Raw Data	2020-07-28	2020-07-28	2020-07-28
Final Report	2020-07-28	2020-07-28	2020-07-28

Quality Assurance Unit: Ou Tingting 2020-07-28

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	2020-06-23
Protocol Effective Date	2020-06-30
Technical Initiation Date	2020-06-30
Technical Completion Date	2020-07-10
Final Report Completion Date	2020-07-29

Edited by: Wang Deheng 2020-07-26
Date

Reviewed by: Zhang Linxi 2020-07-29
Study Director Date

Approved by: Wang Yijie 2020-07-29
Authorized Signatory Date

Sanitation & Environment Technology Institute, Soochow University

Summary

1 Test Article

Test Article Name	Disposable Nitrile Examination Glove
Manufacturer	Shanxi Hongjin Plastic Technology Co., Ltd
Address	Coal Bed Gas Industrial Zone, Qu'e Town, Daning County, Linfen City, Shanxi Province
Model	Not supplied by sponsor (N/S)
Lot/Batch	N/S

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-M202003265-1.

4 Conclusion

Under the conditions of this study, the test article extract showed 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	Disposable Nitrile Examination Glove
Manufacturer	Shanxi Hongjin Plastic Technology Co., Ltd
Address	Coal Bed Gas Industrial Zone, Qu'e Town, Daning County, Linfen City, Shanxi Province
Test Article Initial State	Not Sterilized
CAS Code	N/S
Model	N/S
Size	M
Lot/Batch	N/S
Test Article Material	nitrile
Packaging Material	N/S
Physical State	pieces
Color	blue
Density	N/S
Stability	N/S
Solubility	N/S
Storage Condition	Room Temperature
Intended Clinical Use	to prevent cross contamination

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: Solid
Color: White

4.2.3 Blank Control

Blank Control Article Name: MEM medium, with addition 10% FBS
Physical State: Liquid
Color: Pink
Storage Condition: 4 ± 2°C

5 Equipment and Reagents

5.1 Equipment

Equipment Name	Equipment Number	Calibration Expire
Constant temperature vibrator	SDWH2109	2020-10-28
Autoclave	SDWH2204	2021-03-25
Steel straight scale	SDWH463	2020-07-29
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	MKCD8033
FBS	CORNING	35081006

MEM	HyClone	AF29479404
Trypsin	GiBco	1981725
Penicillin, Streptomycin sulfate	GiBco	2086794
PBS	GiBco	8119427
99.9%Isopropanol	Sinopharm Chemical Reagent Co., Ltd	20191104

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 Sterilization for test samples

Autoclaving at 121°C for 30 min

8.1.1.2 Sterilization for control samples

Same as the test sample.

8.1.2 Extraction

Under aseptic conditions, samples were taken according to the sampling method (Random sampling), and extracted 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.

Test Period	Actual Sampling	Extract Procedure			Final Extract
		Extract Ratio	Volume of Extraction Vehicle	Condition	
Test	120 cm^2	6 cm^2 : 1 mL	20.0 mL	37°C, 24 h	Clear
Negative Control	30 cm^2	3 cm^2 : 1 mL	10.0 mL	37°C, 24 h	Clear
Blank Control	/	/	10.0 mL	37°C, 24 h	Clear
Positive Control	0.5 g	1.0 g:100 mL	50.0 ml	37°C, 24 h	Not Clear

The state of the extract did not change after extraction. The extract was without the process of adjusting its pH value, filtering, centrifuging, diluting, etc. The extract of positive control was filtered before use.

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°C 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°C,>90%humidity) for 24 h. 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°C 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°C 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 35.2%. See Appendix I, 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.

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

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

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

8.6 Evaluation Criteria

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.

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 showed 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			Most cells were round or lysed; cell layers not completely destroyed, cell growth was inhibited.
75% Test article extract			Most cells were round or lysed; cell layers not completely destroyed, cell growth was inhibited.
50% Test article extract			Most cells were round or lysed; cell layers not completely destroyed, cell growth was inhibited.
25% Test article extract			Some cells were round, discrete intracytoplasmatic granules and cell lysis; cell growth was inhibited.

Table 2 Results of the Cell Vitality

Group	Value of OD Mean±SD	Cell Vitality %
Blank control	0.9077±0.035	100.0
Negative control	0.8646±0.022	95.3
Positive control	0.3916±0.037	43.1
100% Test article extract	0.3192±0.022	35.2
75% Test article extract	0.3162±0.020	34.8
50% Test article extract	0.3528±0.028	38.9
25% Test article extract	0.3824±0.036	42.1

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