



中国认可
国际互认
检测
TESTING
CNAS L13034



In Vitro Cytotoxicity Test

MTT Method

Final Report



Verification

Report Number: CSTBB20080832
Article Name: Medical Surgical Mask
Method Standard: ISO 10993-5: 2009

Sponsor

Guangzhou Zhengkang Medical
Equipment Co.,Ltd.

Room 101, No. 9 , Zhongxintang Road,
Nanling, Taihe Town, Baiyun District,
Guangzhou City, China

Test Facility

CCIC Huatongwei international inspection
(Suzhou) Co., Ltd

Room 101, Building G, Ruoshui Road 388,
Suzhou, Jiangsu, China

CCIC Huatongwei international inspection (Suzhou) Co., Ltd

Address: Room 101, Building G, Ruoshui Road 388, Suzhou, Jiangsu, China, 512123 Tel: 0512-87657288 Fax: 0512-87657288

CONTENTS

Notices.....	3
Abstract.....	4
Study Verification and Signature.....	5
Quality Assurance Statement and GLP Statement.....	6
1.0 Purpose.....	7
2.0 Reference.....	7
3.0 Test and control articles.....	7
4.0 Identification and justification of test system.....	7
5.0 Equipment and reagents.....	8
6.0 Experiment design and dose.....	8
7.0 Statistical method.....	9
8.0 Evaluation criteria.....	9
9.0 Results of the test.....	9
10.0 Conclusion.....	10
11.0 Compliance.....	10
12.0 Record.....	10
13.0 Confidentiality Agreement.....	10

Notices

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 report is only responsible for the test results of the tested samples.
5. The report shall not be reproduced except in full without the written approval of the company.



Abstract

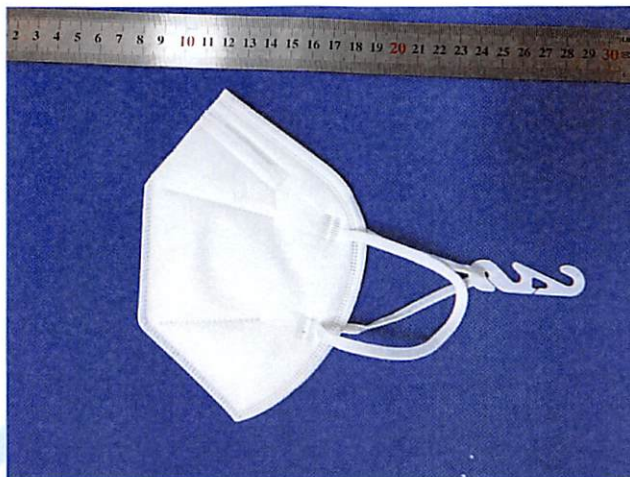
In this study, mammalian L-929 cells were cultured in vitro according to ISO 10993-5:2009 to test the potential cytotoxicity of the test article.

The test articles and the control material were separately placed in MEM medium containing 10% fetal bovine serum, and extracted in a 37 °C incubator for 24 hours. After the end of the extraction, the cell culture medium in the 96-well plate (10^4 cells/well) cultured for 24 hours was removed and replaced with the corresponding extract, cultured in 37 °C, 5% CO₂, >90% humidity for 24 hours. After the culture, the morphology and cell lysis of the cells were observed under the microscope, and the cytotoxicity of the test samples was determined by MTT assay.

The results showed that the cells in the blank control group and the negative control group (high density polyethylene) were well-formed throughout the experiment and showed no cytotoxic reaction. A severe cytotoxic response was shown in the positive control group (ZDEC). The 100% concentration of the test extract retained a normal appearance after 24 hours of incubation, and the cell viability was 87.3%. The data of each group met the acceptance criteria, and the results of this test were valid.

Based on the above results, it can be concluded that under the experimental conditions, the test article Medical Mask have no potential toxicity to L-929 in the MTT method.

Study Verification and Signature



Protocol Number	SST2008023005BB
Protocol Effective Date	2020-08-24
Technical Initiation Date	2020-08-31
Technical Completion Date	2020-09-02
Final Report Completion Date	2020-10-13

Personnel

Betty2020-10-13
Date Completed

Approved

Xing my
Study Director2020-10-13
Date Completed

Supervisory

Jing
Test Facility Manager2020-10-13
Date Completed**CCIC Huatongwei international inspection (Suzhou) Co., Ltd.**

Quality Assurance Statement and GLP Statement

Quality Assurance Statement

The Quality Assurance Unit conducted inspections on the following dates. The findings were reported to the Study Director and to the HTW's Management.

The final report was reviewed to assure that the report accurately describes the methods and standard operating procedures. The reported results accurately reflect the raw data of the nonclinical study conducted per the protocol.

Phase Inspected	Date	Study Director	Management
Experiment	2020-08-31	2020-08-31	2020-08-31
Raw Data	2020-09-02	2020-09-02	2020-09-02
Final Report	2020-10-13	2020-10-13	2020-10-13

The findings of these inspections have been reported to Management and the Study Director.

Hui Fang
Quality Assurance

2020-10-13
Date

GLP Statement

This study was conducted in compliance with current U.S. Food and Drug Administration regulations set forth in 21 CFR, Part 58.

The sections of the regulations not performed by or under the direction of HTW, exempt from this Good Laboratory Practice Statement, included characterization and stability of the test article and its mixture with carriers, 21 CFR, Part 58.105 and 58.113.

Xing Wang
Study Director

2020-10-13
Date

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 Reference

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)

3.0 Test and control articles

Groups	Test article	Negative Control Article	Positive Control Article	Blank Control
Name	Medical Surgical Mask	High Density Polyethylene Film	ZDEC	MEM medium, with addition 10% FBS
Manufacture	Guangzhou Zhengkang Medical Equipment Co.,Ltd.	Hatano Research Institute. FDSC	Sigma-Aldrich.	Hyclone
Size	Not provided	3 cm×10 cm (5 sheets)	25 g	500 ml
Model	ZKM-02	/	/	/
Lot Batch#	2020080502	C-161	BCBQ6847V	AF29479404
Test Article Material	Spun-bond Polypropylene、Melt-blown Polypropylene、PP	/	/	/
Physical State	Solid	Solid	Solid	Liquid
Color	White	White	White	Pink
Packaging Material	polybag	/	/	/
Sterilized or Not	No	No	No	Yes
Concentration	/	/	0.1%	/
Total Surface or weight	Not provided	/	/	/
Storage Condition	Room Tep.	Room Tep.	Room Tep.	4°C
Note: The information about the test article was supplied by the sponsor wherever applicable.				

4.0 Identification and justification of test system

L-929 mouse fibroblast cells obtained from American Type Culture Collection (ATCC).

L-929 cells have been used for cytotoxicity studies because they demonstrate sensitivity to extractable cytotoxic articles. Also, the test article is extracted and administered in vitro to mouse fibroblast L929 cells through a solvent compatible with the test system, which is the optimal route of administration available in this test system as recommended in ISO 10993-5.

5.0 Equipment and reagents

5.1 Instruments

Vertical pressure steam sterilizer (SHB026), CO₂ Incubator (SHB002), Steel Straight Scale (SHB076), Electronic Balance (SHB016), Clean bench (SHB014), Multiskan Spectrum Microplate Spectrophotometer (SHB003), Bench type low speed centrifuge (SHB022), Inverted microscope (SHB005)

5.2 Reagents

MEM (Hyclone, AF29479404), FBS (Clark, JC65941), Penicillin-Streptomycin (Gibco, 2145469), Trypsin (Gibco, 2085461), PBS (Hyclone, AE27749269), MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenylethanolium bromide) (Sigma, MKBG2038V), Isopropyl alcohol (Macklin, C10954717)

6.0 Experiment design and dose

6.1 Sample preparation

According to the table below, aseptic extraction of the test article sealed and incubated in MEM medium (10% FBS) at 37 °C, 5% CO₂ and 60 rpm for 24 hours.

Groups	Sampling		Sterilization	Aseptic Extraction In Inert Container				Final Extract
	Sampling Manner	Actually sampling	Method	Ratio	Extracts	Condition	pH	Clear or Not
Test article	Whole	379.9 cm ²	EO	6 cm ² : 1 ml	63.3 ml	37 °C 24 h	7.4	Clear
Negative Control	Random	60.0 cm ²	UV	3 cm ² : 1 ml	20.0 ml	37 °C 24 h	7.4	Clear
Positive Control	Random	0.02 g	Filter	0.1 g: 100 ml	20.0 ml	37 °C 24 h	7.4	Clear
Blank Control	/	/	/	/	20.0 ml	37 °C 24 h	7.4	Clear

The changes of the leaching solution was observed after extraction. No particulates or color changes were observed in pre- and post-extraction, and immediately be used in the follow-up experiment. The color and pH of the extraction solution did not change before and after use, and the pH value was 7.4 after leaching.

6.2 Test method

Aseptic procedures were used for handling cell cultures. L-929 cells were cultured in MEM medium (10% FBS, 1% Penicillin-Streptomycin solution) at 37 °C in a humidified atmosphere of 5% CO₂, then digested by 0.25% trypsin containing EDTA to get single cell suspension. 1×10^5 cells/ml suspension were obtained by centrifuging (1000 rpm, 5 min) and re-dispersing in MEM medium.

The suspended cells were dispensed at 100 µl per well in 96-well plate, and cultured in cell incubator (5% CO₂, 37 °C, >90% humidity). Cell morphology was evaluated to verify that the monolayer was satisfactory.

After the cells grew to about 70% and 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 and positive article respectively. The 96-well plate was incubated at 37 °C in cell incubator of 5% CO₂ for 24 h. Six replicates of each test were tested.

After incubation, observe the cell morphology first and then discard the culture medium. Add 50 μ l MTT (1mg/ml) to each well and then incubated at 37 °C in a humidified atmosphere of 5% CO₂ for 2 hours. The liquid in each well was tipped out and 100 μ l Isopropyl alcohol 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.

7.0 Statistical method

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

The cell cytotoxicity ratio = OD₅₇₀ of test (or positive or negative) article group/ OD₅₇₀ of blank control group \times 100%.

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

9.1 Results of the cell morphology

Table 1 Observation of the cell morphology

Group	Before 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			The cells showed a round shape and a change in cell morphology occasionally, and there were particles in the cytoplasm, occasionally cell lysis and slight growth inhibition.
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.

9.2 Results of the cell vitality

Table2 Results of the cell vitality

Group	OD value								Viab. (%)
	1	2	3	4	5	6	\bar{x}	s	
Blank control	0.626	0.615	0.618	0.627	0.629	0.629	0.624	0.006	100.0
Negative control	0.619	0.627	0.612	0.601	0.607	0.604	0.612	0.010	98.0
Positive control	0.058	0.059	0.052	0.055	0.060	0.055	0.056	0.003	9.1
100% test article extract	0.533	0.540	0.550	0.541	0.556	0.548	0.545	0.008	87.3
75% test article extract	0.575	0.575	0.571	0.574	0.568	0.578	0.573	0.004	91.9
50% test article extract	0.591	0.607	0.610	0.610	0.608	0.606	0.605	0.007	97.0
25% test article extract	0.594	0.596	0.594	0.606	0.601	0.607	0.600	0.006	96.1

10.0 Conclusion

Under the conditions of this study, the test article have no potential toxicity to L-929 cells.

11.0 Compliance

US FDA Good Laboratory Practice Regulations 21 CFR 58, effective June 20, 1979, as amended 52 FR 33780, Sept. 4, 1987, and subsequent amendments

Standard operating procedure of CCIC Huatongwei international inspection (Suzhou) Co., Ltd.

12.0 Record

All raw data pertaining to this study and a copy of the final report are to be stored in the designated archive files at Huatongwei.

13.0 Confidentiality Agreement

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