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CNAS L10463

FINAL REPORT

Study Name: Disposable Medical Face Masks- In Vitro Cytotoxicity

Test

Study Number: MED202008586-01-EN



Sponsor

Name: Changzhou Huankang Medical Device Co., Ltd.

Address: 22 Changhe Road, Changzhou, Jiangsu, China

Testing Facility

Name: EPIN Suzhou Ltd.

Address: No.558 Fenhu Avenue, Lili Town, Wujiang District, Suzhou, China

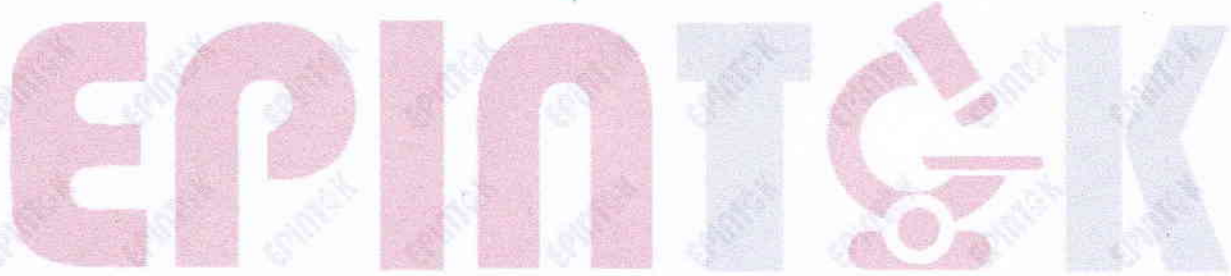


SUPPLEMENTARY EXPLANATION

1. Please apply for rechecking within 15 days after receiving the report if there is any objection.
2. The report is only valid with the dated signatures by person responsible and cross-page seal.
3. The results in this report relate only to the article tested.
4. The test report shall not be reproduced except in full, without written approval of EPIN Suzhou Ltd.
5. ILAC-G8:09/2019 was employed as the decision rules of statement conformity, where applicable.



TEST ARTICLE CONFIRMATION AND SIGNATURE



Edited by: Arain Yan
Arain Yan (Report Drafter)

Date: 2020-09-21

Checked by: Alice Wang
Alice Wang (Study Director)

Date: 2020-09-21

Approved by: Julie Wang
Julie Wang (Authorized Signatory)

Date: 2020.09.21



TABLE OF CONTENTS

1. STUDY SUMMARIES 6

1.1. Study Name (Study No.)..... 6

1.2. Purpose 6

1.3. Referred Standard 6

1.4. Testing Facility 6

1.5. Sponsor 6

1.6. Study Alteration Treatment..... 6

1.7. Deviation(s) and Incident(s) Treatment 6

1.8. Major Laboratory Personnel(s) 7

1.9. Schedule of the Study 7

2. TEST MATERIAL..... 7

2.1. Test Article 7

2.1.1. General information¹⁾..... 7

2.1.2. Retention of test article(s)..... 8

2.1.3. Handling of residual test article(s)..... 8

2.2. Blank Control..... 8

2.3. Negative Control..... 8

2.4. Positive Control 9

2.5. Main Instruments and Reagents..... 9

2.5.1 Main instruments 9

2.5.2 Main reagents..... 9

3. JUSTIFICATION OF THE TEST SYSTEM..... 9

4. IDENTIFICATION OF TEST SYSTEM..... 10

5. ROUTE OF ADMINISTRATION..... 10

6. TEST DESIGN..... 10

6.1. Extract Preparation..... 10

6.2. MTT Solution Preparation 10

6.3. Experimental Process..... 10

7. DATA ANALYSIS 11

8. EVALUATION CRITERION..... 11

9. ALTERATION AND DEVIATION..... 11

10. RESULTS 11

10.1. Results of Cell Morphology..... 12

10.2. Results of Cell Vitality..... 12

11. CONCLUSION 13

12. ARCHIVING 13

SUMMARY

1. Purpose

The purpose of the test is to determine the biological reactivity of a mammalian cell culture (mouse fibroblast L-929 cells) in response to the test article Disposable Medical Face Masks.

2. Process Description

The suspended cells were dispensed in 96-well plate, and cultured it in cell incubator (5% CO₂, 37°C) on the first day.

On the second day, the test article extract (100%, 75%, 50% and 25% in growth medium) was added to L-929 cells in 96-well plates and then incubated at 37°C in 5% CO₂ for another 24 h.

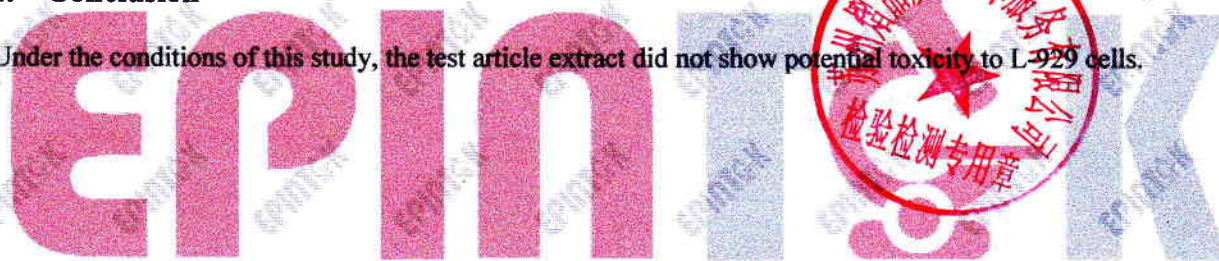
After 24 h incubation, observed the cell morphology first and 50 µL aliquot of MTT (1 mg/mL) was added. 2 h incubation later, determined the OD value.

3. Result

The MTT method results showed that the cytotoxicity ratio of the 100% test article extract was 86.7%. The results of control groups showed the test was valid.

4. Conclusion

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



1. STUDY SUMMARIES

1.1. Study Name (Study No.)

Disposable Medical Face Masks - In Vitro Cytotoxicity Test (MED202008586-01-EN)

1.2. Purpose

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

1.3. Referred Standard

- 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

1.4. Testing Facility

Name: EPIN Suzhou Ltd.
Address: No.558 Fenhu Avenue, Lili Town, Wujiang District, Suzhou, China

1.5. Sponsor

Name: Changzhou Huankang Medical Device Co., Ltd..
Address: 22 Changhe Road, Changzhou, Jiangsu, China
ATTN: Yecheng Zhai
Contact Information: +86 150 2166 5265/519 8890 9800/hk@huankang.com

1.6. Study Alteration Treatment

Before the study start, the study protocol was approved by Study Director and Sponsor. Any study alteration should be approved by Study Director.

1.7. Deviation(s) and Incident(s) Treatment

If any deviation or incident occurred during the test, the related information would be recorded timely and a deviation report should be submitted with the final report to interpretate the specific effect(s) on the final result caused by the deviation or incident.

1.8. Major Laboratory Personnel(s)

Study Director: Alice Wang
 Main Operation Personnels: Alice Wang, Arain Yan

1.9. Schedule of the Study

Test Article Received Date: 2020-08-26
 Protocol Effective Date: 2020-08-31
 Technical Initiation Date: 2020-09-09
 Technical Completion Date: 2020-09-11
 Report Signature Date: 2020-09-21

2. TEST MATERIAL

2.1. Test Article

2.1.1. General information¹⁾

Name: Disposable Medical Face Masks
 Initial State: Sterile, EO
 Size: 175*95mm
 Model: HK-Z01
 Lot/ Batch#: 20200820
 Physical State: Solid
 Color: N/S
 Density: N/S
 Stability: N/S
 Solubility: N/S
 Storage Condition: Room temperature
 Test Article Material: N/S
 Packaging Material: N/S
 Manufacturer Name: Changzhou Huankang Medical Device Co., Ltd..

Manufacturer Address: 22 Changhe Road, Changzhou, Jiangsu, China

- 1) The information about the test article was supplied by the sponsor wherever applicable.
- 2) N/S means not supplied by the sponsor.

2.1.2. Retention of test article(s)

Retention Volume: 10 pcs
Retention Location: Sample reserve room

2.1.3. Handling of residual test article(s)

Tested Article(s): Destroy and Waste
Untested Article(s): Destroy and Waste

2.2. Blank Control

Name: MEM medium with 10% FBS
Manufacturer: Hyclone
Size: 500 mL
Lot/ Batch#: AF29494682
Physical State: Liquid
Color: PINK
Storage Condition: (2-8) °C

2.3. Negative Control

Name: High Density Polyethylene
Manufacturer: U.S.Pharmacoopia
Size: 3 Strips
Lot/Batch#.: KOM357
Physical State: Solid
Color: White
Storage Condition: Room temperature
Extract Ratio: 3 cm²: 1 mL
Extract Condition: 37°C, 24 h

2.4. Positive Control

Name: Zinc diethyldithiocarbamate
 Manufacturer: Aladdin
 Size: 25 g
 Lot/Batch#: K1301032
 Physical State: Solid
 Color: White
 Storage Condition: (2-8) °C
 Final Concentration: 1%
 Extract Condition: 37°C, 24 h

2.5. Main Instruments and Reagents

2.5.1 Main instruments

Name	No.	Calibration Due Date
CO ₂ Incubator	EPB-122	2020-10-20
Shaking Water Bath	EPB-232	2021-05-08
Inverted Microscope	EPB-003	2020-10-20
Micro-plate Reader	EPB-192	2021-04-12

2.5.2 Main reagents

Name	Lot/ Batch#	Source
3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT)	C10769615	PERFEMIKER
Fetal bovine serum (FBS)	2064652	Gibco
Minimum essential medium (MEM)	AF29494682	Hyclone
Penicillin streptomycin sulfate	J190015	Hyclone
Trypsin	J190034	Hyclone
Isopropanol	20200313	SCR

3. JUSTIFICATION OF THE TEST SYSTEM

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

4. IDENTIFICATION OF TEST SYSTEM

L-929 mouse fibroblast cells obtained from Shanghai Cell Line Resources, CAS.

5. ROUTE OF ADMINISTRATION

The test article was extracted and administrated 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 guidelines.

6. TEST DESIGN

6.1. Extract Preparation

Table 1 Extraction process

Aseptically Sampling			Solvent	Sterilization Method	Extract Condition
Ratio	Sampling Manner	Actually Sampling			
3 cm ² /1 mL	Whole	332.5 cm ² (provided by sponsor)	MEM with 10% FBS	/	37°C 24 h

Table 2 Final extract¹⁾

Extract	pH	Clear or Not	Color changed or not	Presence of particles or not	Diluted/ filtered/pH adjusted or not
110.83 mL	8.0	Clear	Not	Not	Not

1) The final extracts used immediately.

6.2. MTT Solution Preparation

100 mg MTT powder was dissolved in 100 mL PBS. Then sterilized by filtering with 0.22 μm membrane and kept in the dark at 4°C for one week.

6.3. Experimental Process

Aseptic procedures were used for handling of cell cultures.

L-929 cells were cultured in MEM medium (10% FBS, Penicillin 100 U/mL, Streptomycin 100 μg/mL) at 37°C in a humidified atmosphere of 5% CO₂, then digested with 0.25% trypsin to get single cell suspension. And obtained a 1×10⁵ cells/mL suspension by centrifuging (1000 rpm, 5 min) and re-suspended in MEM medium finally.

The suspended cells were dispensed at 100 μL per well in 96-well plate, and cultured it in cell incubator (5%

CO₂, 37°C), 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 extract of test article (100%, 75%, 50%, 25%), blank control, negative control (100%) and positive control (100%) respectively. Incubated the 96-well plate incubator (37°C, 5%CO₂) for another 24 h. Six parallel wells of each test were tested.

After 24 h incubation, firstly observed the cell morphology and then discarded the culture medium. 50 µL aliquot of MTT (1 mg/mL) was added to each well and then incubated at 37°C in a humidified atmosphere of 5% CO₂ for 2 h. The liquid in each well was tipped out and 100 µL isopropanol was added to each well.

Evaluated the OD Value with a dual-wavelength spectrophotometer with the measurement wavelength at 570 nm and reference wavelength at 650 nm.

7. DATA ANALYSIS

Mean±standard deviation ($\bar{X} \pm SD$)

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

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

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

8. EVALUATION CRITERION

- The lower the Viab.% value, the higher the cytotoxic potential of the test article is.
- Reduction of cell viability by more than 30 % is considered a cytotoxic effect.

9. ALTERATION AND DEVIATION

Alteration and deviation did not happen in this study.

10. RESULTS

10.1. Results of Cell Morphology

Table 3 Observation of 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.
Negative control			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.
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.	Discrete intracytoplasmatic granules, no cell lysis, no reduction of cell growth.	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.
75% Test article extract			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.
50% Test article extract			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.
25% Test article extract			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.

10.2. Results of Cell Vitality

Table 4 Results of cell vitality

Group	$\bar{X} \pm SD$	Viability%
Blank control	0.314±0.006	100.0%
Negative control	0.280±0.006	89.3%
Positive control	0.013±0.002	4.0%
100% test article extract	0.272±0.004	86.7%
75% test article extract	0.274±0.009	87.2%
50% test article extract	0.274±0.004	87.5%
25% test article extract	0.260±0.017	82.9%

11. CONCLUSION

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

12. ARCHIVING

All correspondence, including original copy of the protocol, original copy of the test report, and all raw data generated during the study (i.e., documentation forms as well as any other notes of raw data, printouts of instruments and computers) are stored in the archives room of the EPIN Suzhou Ltd.

..... End of the Report

