FINAL REPORT

Study Name：IVD Reagent Bottle - In Vitro Cytotoxicity Test

Study Number: MED201706054-01

Testing Facility

Name：Epin Suzhou Ltd.

Address：Building 4, No.558 Fenhu Avenue, Wujiang District, Suzhou, China

Sponsor

Name：Shang Hai Jun Cheng Biotechnology Co.,Ltd

Address：Jiang Gao Road 420 Medical New&Hi-Tech Industrial Deveopment Zone Tai zhou Jiangsu China

Manufacturer

Name：Shang Hai Jun Cheng Biotechnology Co.,Ltd

Address：Jiang Gao Road 420 Medical New&Hi-Tech Industrial Deveopment Zone Tai zhou Jiangsu China

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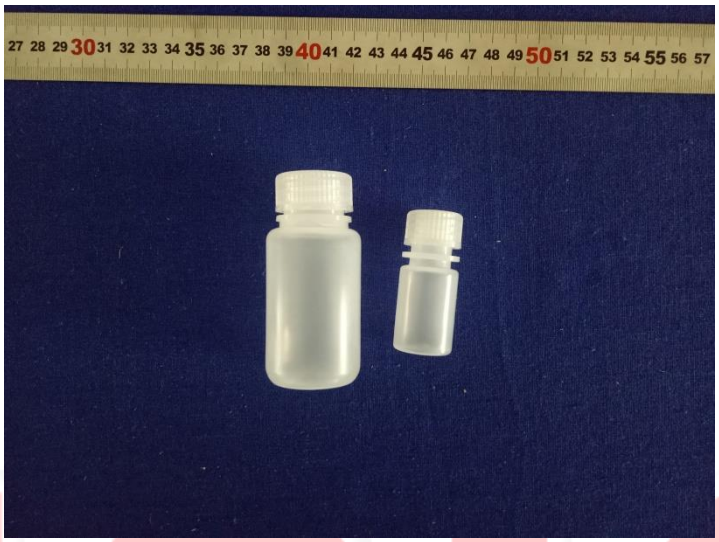

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SUMMARY

1. Purpose

The purpose of the test is to assess the potential cytotoxicity of test article IVD Reagent Bottle in the In Vitro Cytotoxicity Test using L929 mouse fibroblast cells (From ATCC), which was sensitive to extractable cytotoxic articles.

2. Experimental Process

The suspended cells were dispensed in 96-well plate, and cultured it in cell incubator (5% CO_2，37℃) 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℃ in 5% CO_2 for another 24h.

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

3. Result

The MTT method results showed that the cytotoxicity ratio of the 100% test article extract was 110.7%.

The results of control groups showed the test was valid.

4. Evaluate

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.)

In Vitro Cytotoxicity Test (MED201706054-01)

1.2. Study 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. Guide

☒ 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: Building 4, No.558 Fenhu Avenue, Wujiang District, Suzhou, China

1.5. Sponsor

Name: Shang Hai Jun Cheng Biotechnology Co.,Ltd.

Address: Jiang Gao Road 420 Medical New&Hi-Tech Industrial Deveopment Zone

Tai zhou Jiangsu China

1.6. Study Alteration Treatment

Before the study start, the test protocol was approved by Study Director and Facility Manager. Study alteration did not happen.

1.7. Deviation And Accident Treatment

If deviation or accident occured during the test, the related information would be recorded timely and deviation report should be submitted with the final report to interpretate the specific effect on the final result caused by the deviation or accident.
Deviation and accident did not happen in this study.

1.8. Major Laboratory Personnel

Study Director：	Julie Wang
Main Operation Personnel：	Alice Wang

1.9. Schedule of the Study

Protocol effective date：	2017-07-27
Technical initiation date：	2017-07-27
Technical completion date：	2017-07-29
Report signature date：	2017-08-01

2. TEST MATERIAL

2.1. Test Article

Name：	IVD Reagent Bottle
Initial state：	Not Sterilized
CAS/Code#：	Not Supplied by Sponsor (N/S)
Size：	N/S
Model：	N/S
Lot/ Batch#：	N/S
Physical State：	Solid
Color：	Natural
Density：	0.91
Stability：	N/S
Solubility：	N/S
Storage Condition：	Room temperature
Test article material：	PP
Packaging Materia：	PE bag

Note：The information about the test article was supplied by the sponsor wherever applicable.

2.2. Vehicle

Name：	MEM medium, with addition 10% FBS

Manufacturer：Hyclone

Size：500mL

Lot/ Batch#：AC10203423

Physical State：Liquid

Color：Pink

Storage Condition：(4±2) ℃

2.3. Negative Control

Name：High Density Polyethylene

Manufacturer：U.S.Pharmacopeia

Size：3 Strips

Lot/Batch#.：K0M357

Physical State：Solid

Color：White

Storage Condition：Room temperature

Extract ratio：$3cm^2$:1mL

2.4. Positive Control

Name：Zinc diethyldithiocarbamate

Manufacturer：Aladdin

Size：25g

Lot/Batch#.：K1301032

Physical State：Solid

Color：White

Storage Condition：(4 ± 2) ℃

Final Concentration：1%

2.5. Main Instruments And Reagents

2.5.1 Instruments

Name	No.	Manufacturer
CO_2 Incubator	EPB-001	Shanghai Shencheng technology Co., Ltd.

Automatic counting cell meter	EPB-002	Logos biosystems
Inverted microscope	EPB-003	Shanghai optical instrument factory No. 6
Micro-plate reader	EPB-017	Shanghai Kehua test system Co., Ltd.

2.5.2 Reagents

Name	Lot/ Batch#	Manufacturer
MTT	0793	PERFEMIKER
FBS	1698221	Gibco
MEM culture medium	AC10203423	Hyclone
Trypsin	J150050	Hyclone
Penicillin Streptomycin sulfate	J160007	Hyclone
99.9% Isopropanol	C10090993	Macklin

3. JUSTIFICATION OF THE TEST SYSTEM

Historically, mouse fibroblast L929 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 ATCC (American Type Culture Collection), USA.

5. ROUTE OF ADMINISTRATION

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.

6. TEST DESIGN

6.1. Extract Preparation

Aseptically extracted the test article by MEM medium with 10% FBS according to the table below and Incubated at 37℃ and 5% CO_2 for 24h. Extract was used immediately after extraction.

Aseptically Sampling			Sterilization Method	Final Extract		
Ratio	Sampling Manner	Actually sampling		Extracts	pH	Clear or Not

0.2g:1mL	Whole	6.85g	UV irradiation	34.25mL	7.4	Clear

The blank control, negative control and positive control were prepared in the same way.

6.2. MTT Solution Preparation

Took 32mg MTT powder and dissolved in 32mL PBS buffer solution. Then sterilized by filtering with 0.45μm membrane and kept in 4℃ avoid light for one week.

6.3. Experimental Process

Aseptic procedures were used for handling of cell cultures.

L929 cells were cultured in MEM medium (10% FBS, Penicillin 100 U/mL, Streptomycin 100 U/mL) at 37℃ in a humidified atmosphere of 5% CO_2, then digested with 0.25% trypsin to get single cell suspension. And obtained a 1×10^5 cells/mL suspension by centrifuging (1000rpm, 5min) and re-suspended in MEM medium finally.

The suspended cells were dispensed at 100μL per well in 96-well plate, and cultured it in cell incubator (5% CO_2，37℃), 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 extract of test article (100%, 75%, 50%, 25%), blank control, negative control (100%) and positive control (100%) respectively. Incubated the 96-well plate at 37℃ in cell incubator of 5% CO_2 for another 24h. Six parallel wells of each test were tested.

After 24h incubation, firstly observed the cell morphology and then discarded the culture medium. 50μL aliquot of MTT (1mg/mL) was added to each well and then incubated at 37℃ in a humidified atmosphere of 5% CO_2 for 2h. The liquid in each well was tipped out and 100μL 99.7% 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（$\bar{X}+\mathrm{SD}$）

The cell cytotoxicity ratio = [$\overline{OD_{570}-OD_{650}}$] of test (or positive and negative) article group/ [$\overline{OD_{570}-OD_{650}}$] of blank control group×100%.

8. EVALUATION CRITERION

- The lower the Viab.% value, the higher the cytotoxic potential of the test item is.
- If viability is reduced to < 70 % of the blank, it has a cytotoxic potential.
- The 50 % extract of the test sample should have at least the same or a higher viability than the 100 % extract; otherwise the test should be repeated.

9. RESULTS

9.1 Results of Cell Morphology

Table 1 Observation of cell morphology

Group	Before inoculation	Before treated with extract	24h 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.

9.2 Results of Cell Vitality

Table 2 Results of cell Vitality

Group	$\overline{X} + SD$	Viability%
Blank control	0.468±0.059	100.0%
Negative control	0.458±0.045	97.8%
Positive control	0.015±0.001	3.2%
100% test article extract	0.519±0.045	110.7%
75% test article extract	0.543±0.035	115.8%
50% test article extract	0.558±0.036	119.2%
25% test article extract	0.489±0.035	104.4%

10. CONCLUSION

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

11. ARCHIVING

All correspondence, an original copy of the protocol, an original copy of the test report, and a documentation of all raw data generated during the conduct of 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 of the Epin Suzhou Ltd.

Epin Suzhou Ltd. will archive the study file for 6 years. The sponsor shall give an official application to Epin Suzhou Ltd. for the extended archiving period. Otherwise all documents and materials may be destroyed by Epin Suzhou Ltd. at the end of the archiving period.

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