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CNAS L9783

**Guangdong Neway Quality Technology Service Co., Ltd.**

# Test Report

***In Vitro* Cytotoxicity Test ISO 10993-5:2009**

**（Direct contact Method）**

**Report No.: WT24032230-7**

Test Sample: **Food grade & medical grade silicone oil**

Sponsor: Shandong Silico Silicone Materials Co., Ltd.

Manufacturer: Shandong Silico Silicone Materials Co., Ltd.

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# ABSTRACT

Sponsor / Client: Shandong Silico Silicone Materials Co., Ltd.

Address: No. 3168, Daiyue Economic Development Zone, Tai'an City, Shandong Province, China

Manufacturer: Shandong Silico Silicone Materials Co., Ltd.

Address: No. 3168, Daiyue Economic Development Zone, Tai'an City, Shandong Province, China

Test Sample: Food grade & medical grade silicone oil, MEM (containing 10% fetal bovine serum) was used to perform in vitro cytotoxicity test according to the methods recommended in the standard ISO 10993-5:2009. The appropriate samples were put into the culture medium, and the corresponding blank, Filter paper, negative and positive control groups were likewise put into the culture medium, and after 24 hours, the cell morphology was observed under the microscope to qualitatively grade the cytotoxicity morphology.

| | |
|---|---|
| **Conclusion:** | Under the conditions of this test, the cytotoxic reaction of the test sample was grade 1, indicating that the sample was non-cytotoxic to L929 cells. |

Lead Technician: Zhiping Zheng Date: Feb.26,2025

Report Reviewer: Shanglou Liu Date: Feb.26,2025

Authorized Signatory: Honglei Xu Approval Date: Feb.26,2025

Report issued by:

GUANGDONG NEWAY QUALITY TECHNOLOGY SERVICE CO., LTD.

**(Test Stamp)**

# PURPOSE AND SCHEDULE

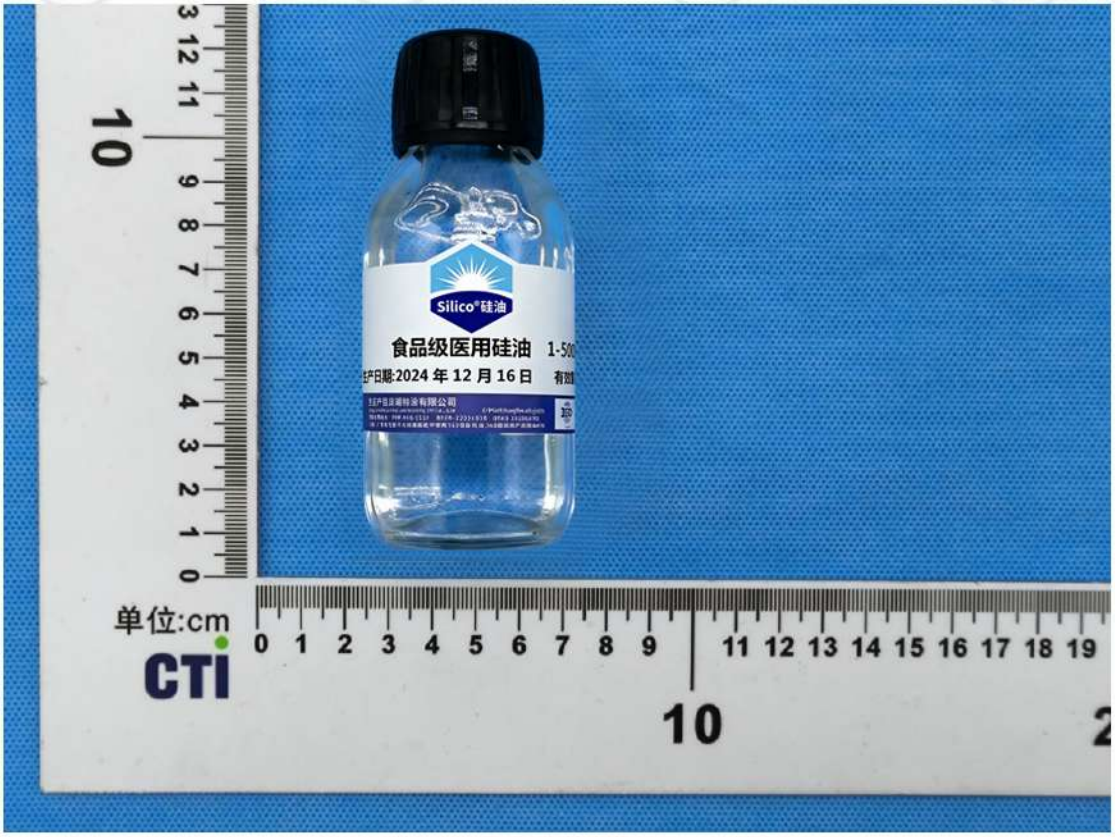
In vitro cytotoxicity test is performed on the samples according to the agar diffusion test recommended in the standard ISO 10993-5:2009 Biological Evaluation of Medical Devices Part 5: In Vitro Cytotoxicity Test.

The purpose of this test is to confirm the degree to which the sample induces a cytotoxic reaction when it passes through the agar layer and comes into contact with the cells.

Study Initiation Date: Dec. 19, 2024

Test Starting Date: Dec. 23, 2024

Test Completion Date: Dec. 25, 2024



# 1. TEST BASIS

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 10993-12:2021)

# 2. MATERIALS INFORMATION

## 2.1 Test Sample

| | |
|---|---|
| Test Sample Description: | Food grade & medical grade silicone oil |
| Major Ingredients: | silicone oil |
| Intended Use: | Medical device lubrication |
| Batch/Lot No.: | 30020241210 |
| Model No.: | BX1-BX5000000 |
| Sample surface area or mass: | Not provided |
| Production Date: | Not provided |
| Expiration Date: | Not provided |
| Storage Condition: | Ambient temperature |
| External Features: | Liquid |
| Sterilization Method: | Non-Sterilized |
| Pre-treatment: | Non |
| Note: The test sample information is provided by the sample client. | |

## 2.2 Reagents

Fetal bovine serum (FBS) (SORFA Lot: SH5021)

0.25% EDTA-trypsin (GIBCO Lot: 2660237)

L-glutamine (Biological Industries Lot: 2227144)

MEM (GIBCO Lot: 2508894)

Penicillin-Streptomycin solution (Biological Industries Lot: 2338304)

HDPE (Hatanto Research Institute Lot: C-231)

Rubber gloves (Medicom Lot: 20240605)

PBS buffer (BIOSHARP Lot: 24149299)

0.4% Trypan blue (BIOSHARP Lot: 24176678)

6 well plate (CELLPRO BIO Lot: MO2024100097)

## 2.3 Equipment

$CO_2$ Incubator (TTF20241140)

Super clean bench (TTE20241139)

Inverted Microscope ( TTE20233876)

Adjustable precision pipette (20-200 μL： TTE20242812)

Adjustable precision pipette (100-1000 μL TTE20242810)

Centrifuge (TTF20241138)

Inverted Microscope (TTE20233876)

Countstar cell counter (TTE20233865)

## 3. SAMPLE AND CONTROLS PREPARATION

Test Sample extract and control preparation were performed under sterile environment with aseptic technique. The sample and control sample preparation are shown in Table 1.

Table 1 Sample and control sample preparation information

| Groups | | Sampling Method | Whether filter paper is needed | Sampling (area) | Sterilization Method |
|---|---|---|---|---|---|
| Test Sample | Food grade & medical grade silicone oil | Random | Yes | 1×1cm$^2$,20μL | Autoclave (121 ℃,20 min) |
| Negative Control | HDPE | Random | / | 1×1cm$^2$ | Soak in 75% alcohol for 30min |
| Blank Control | / | / | / | / | / |
| Filter paper group | Filter paper | Random | Yes | 1×1cm$^2$,20μL | Autoclave (121 ℃,20 min) |
| Positive Control | Rubber gloves | Random | / | 1×1cm$^2$ | Sterilized |

# 4. EXPERIMENTAL DESIGN

## 4.1 Experimental System

4.1.1 Cell Strain: NCTC clone 929 (L929)

4.1.2 Cell Source: CTCC

4.1.3 Sub culturing: Well growing cells subculture for 48 h～72 h

4.1.4 Justification of experimental system: *In vitro* cytotoxicity test is one of the most important tests for medical device biocompatibility evaluation system. NCTC clone 929 Cell is endorsed by ISO experts to be suitable for in vitro cytotoxicity test.

4.1.5 Facility: Experimental facility is accredited in accordance with ISO/IEC 17025 by CNAS (China National Accreditation Service). CNAS registration Number: CNAS L9783.

4.1.6 Personnel: All relevant personnel are well trained and qualified.

4.1.7 Compliance: The study referenced in this report was conducted in compliance with ISO/IEC17025:2017 General requirements for the competence of testing and calibration laboratories and China National Accreditation Service for Conformity Assessment (CNAS) Accreditation criteria for the competence of testing and calibration laboratories, CNAS CL01: 2018, effective September 1st, 2018.

## 4.2 Test Procedure

4.2.1 L-929 cells were cultured in MEM culture medium containing 10% fetal bovine serum and double antibody (penicillin 100 U/mL, streptomycin 100 μg/mL), placed in 37℃, 5% $CO_2$ incubator, cells were digested with 0.25% trypsin, prepared into single-cell suspensions, adjusted the concentration of the cells to $1 \times 10^5$ cells/ml, and the prepared $1 \times 10^5$/ml cell suspension was inoculated into a 6-well culture plate, Filter paper group, negative control, positive control and sample group were set up, each group had 3 parallel wells, and each well was inoculated with 2 ml of cell suspension.

4.2.2 After being cultured in a $CO_2$ incubator (containing 5% $CO_2$ gas by volume, the same below) at 37℃ for 24 hours, the original culture medium was discarded.0.8 mL of fresh MEM medium containing 10% FBS was added to each well of a 6-well culture plate. The

test positions of the sample group, filter paper group, positive control group, negative control group and blank control group were marked. 20 μL of the sample was used to soak the filter paper thoroughly.The filter paper of the filter paper group was soaked thoroughly with fresh complete MEM medium containing 10% FBS.The negative control group and the positive control group were treated in the same way and directly placed in the medium.The blank group was not treated.Each group had 3 parallel wells.They were placed in a 37°C, 5% $CO_2$ incubator for another 24 hours of culture, and then observed under a microscope for cell morphology.

### 4.3 Data Analysis

Cells are examined microscopically and, if necessary, cytochemical staining is used to evaluate alterations such as general morphology, vacuole formation, detachment, cytolysis and cell membrane integrity. Specific methods used for qualitative morphological grading of cytotoxicity of test samples are given in Table 2.

Table 2 Reactivity grades for direct contact test

| Grade | Reactivity | Description of reactivity zone |
|---|---|---|
| 0 | None | No detectable zone around or under specimen |
| 1 | Slight | Some malformed or degenerated cells under specimen |
| 2 | Mild | Zone limited to area under specimen |
| 3 | Moderate | Zone extending specimen size up to 1.0 cm |
| 4 | Severe | Zone extending farther than 1.0 cm beyond specimen |

# 5. TEST RESULTS

5.1 The specific results of this test are as follows (Table 3):

Table 3 Cytotoxic reaction results

| Groups | Serial Number | Grade | Final Score |
|---|---|---|---|
| Negative group | 1 | 0 | 0 |
| | 2 | 0 | |
| | 3 | 0 | |
| Blank group | 1 | 0 | 0 |
| | 2 | 0 | |
| | 3 | 0 | |
| Positive group | 1 | 4 | 4 |
| | 2 | 4 | |
| | 3 | 4 | |
| Sample group | 1 | 0 | 1 |
| | 2 | 0 | |
| | 3 | 1 | |
| Filter paper group | 1 | 0 | 0 |
| | 2 | 0 | |
| | 3 | 0 | |

5.2 The results and conclusions apply only to the samples tested. The testing organisation has not evaluated these results further. The commissioning organisation is responsible for interpreting whether the data are applicable to other samples. All steps were carried out in accordance with the test code of practice.

# 6. DEVIATION

This study was completed in accordance with the protocol, no deviation occurred during the test.

# 7. CONCLUSION

Under the conditions of this test, the cytotoxic reaction of the test sample was grade 1, indicating that the sample was non-cytotoxic to L929 cells.

## 8. ARCHIVING

All the study-related raw data, records, protocol and the final report will be kept in NEWAY for 6 years from report issue date. For studies of more than 4 weeks duration, the test samples will also be in kept in NEWAY for 6 years from report issue date.

***End of Report***

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