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NEWAY TESTING LABORATORY

Testing Report

In Vitro Cytotoxicity Test ISO 10993-5:2009

(MTT Method)

Report No.: WT20010538-4

Test Sample: Conductive Hydrogel

Sponsor: DONGGUAN QUANDING MEDICAL SUPPLIES CO., LTD

Manufacturer: DONGGUAN QUANDING MEDICAL SUPPLIES CO., LTD

Neway TESTING

Remarks

1. The results shown in this test report refer only to the sample(s) tested.
2. The content of this report is invalid if it is not presented as the entire report.
3. Any unauthorized alteration, forgery or falsification of the content or appearance of this report is unlawful and offenders may be prosecuted fully of the law.

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ABSTRACT

Sponsor / Client: DONGGUAN QUANDING MEDICAL SUPPLIES CO., LTD

Address: 3 YONGFA EAST ROAD, QISHI TOWN, DONGGUAN CITY, GUANGDONG, CHINA.

Manufacturer: DONGGUAN QUANDING MEDICAL SUPPLIES CO., LTD

Address: 3 YONGFA EAST ROAD, QISHI TOWN, DONGGUAN CITY, GUANGDONG, CHINA.

Test Sample: Conductive Hydrogel, was extracted with MEM with 10% serum, and subjected to in vitro cytotoxicity test according to Annex C of ISO 10993-5:2009.

Mouse lung fibroblast cells (L929 Cell) are subjected to contact with test sample extract, blank control, negative control and positive control separately. After 24h culturing, measure the OD value of each group and calculate cell viability.

Quality control qualified the reliability of the test results.

Conclusion:	Test Sample induced non-cytotoxicity to L929 cells.

Study Director: _____ Date: Nov. 13, 2020
Liu Shanglou

Report Reviewer: _____ Date: Nov. 13, 2020
Wang Meng

Authorized Signatory: _____ Approval Date: NOV. 13, 2020
Xu Honglei

Report issued by:
GUANGDONG NEWAY QUALITY TECHNOLOGY SERVICE CO., LTD.
(Testing Stamp)

QUALITY ASSURANCE STATEMENT

This test was audited by Quality Assurance (QA) personnel of NEWAY. The QA inspection includes review of study plan, result of a study-based audit and results of audit of raw data and study report.

The findings were reported to Study Director and NEWAY management.

QA manager:

Xu Honglei

NOV. 13. 2020

Date Completed

Inspection Type	Inspection Date	Testing Phase	Date to Facility Manager and Study Director
Study base	Sep. 21, 2020	Draft Protocol	Sep. 21, 2020
Study base	Oct. 21, 2020	Test Sample Preparation	Oct. 21, 2020
Study base	Oct. 23, 2020	Raw Data & Draft Final Report	Oct. 23, 2020

PURPOSE AND SCHEDULE

According to its nature and duration of the anticipated contact with human tissues when in use, medical device should be tested for biocompatibility to avoid potential physiological damage from toxic substances produced or contaminated during manufacturing.

In this study, in vitro cytotoxicity test was performed to evaluate toxicity of substances that could be extracted or released from the medical device. Mouse lung fibroblast cells (L929 cells) are used here as test system for cytotoxicity test. Based on recommendations described in ISO 10993-5, quantitative determination of cell viability by MTT assay and qualitative observation of cell morphology and growth density were carried out. These results provide practical information for assessing the in vitro cytotoxicity of the medical device.

Study schedule as below:

Study Initiation Date: Sep. 21, 2020

Test Starting Date: Oct. 21, 2020

Test Completion Date: Oct. 23, 2020

PHOTO OF TEST SAMPLE

1. MATERIALS INFORMATION

1.1 Test Sample

Test Sample information was provided by sponsor and showed as below:

Test Sample Description:	Conductive Hydrogel
Specify the test parts or materials:	Conductive Hydrogel
Major ingredients:	Conductive Hydrogel
Intended Use:	Conducting electric current to the skin surface or transmitting human EMG or ECG signals to the machine.
Batch/Lot No.:	20200915
Model No.:	QD0.9A
Stability:	Not entrusted
Production date:	20200915
Expiration Date:	20220914
Storage Condition:	Ambient temperature
External Features:	Solid
Sterilization Method:	Non-Sterilized
Pre-treatment:	Non
Extraction Vehicle:	MEM (with 10% FBS)

1.2 Reagents

1.2.1 Phenol (Howei Pharm Lot: 14155174)

1.2.2 MTT (SIGMA Lot: MKCL1832)

1.2.3 Fetal bovine serum (FBS) (HYCLONE Lot: DD19362264)

1.2.4 Trypsin solution (GIBCO Lot: 2152925)

1.2.5 MEM (GIBCO Lot: 2048092)

1.2.6 Penicillin-Streptomycin solution (GIBCO Lot: 2076677)

1.2.7 Isopropanol (Tianjin Zhiyuan Chemical Lot: 20194030118)

1.2.8 HDPE (USOLF Lot: P25141115)

1.3 Equipment

1.3.1 Shaker water bath (Changzhou Lang Yue, K2017-01-01)

1.3.2 CO_2 Incubator (ESCO, K2016-05-01)

1.3.3 Super clean bench (SUJIE PURIFICATION, Z2016-01-01)

1.3.4 Inverted Microscope (OLYMPUS, R2016-02-02)

1.3.5 Adjustable precision pipette (THERMO FISHER, D2016-02-04)

1.3.6 Adjustable precision pipette (THERMO FISHER, D2016-03-04)

1.3.7 Adjustable precision pipette(8 Channels) (THERMO FISHER, D2016-04-04)

1.3.8 Shaker (KYLIN-BELL,J2016-01-01)

1.3.9 Microplate Reader (THERMO FISHER, X2016-02-01)

1.3.10 Centrifuge (LICHEN, X2017-01-01)

1.3.11 Balance (Sartorious, C2016-01-01)

1.4 Test Sample Extraction and Controls Preparation

Test Sample extract and controls preparation were performed under sterile environment with aseptic technique.

Extraction was done in centrifuge tube with cap in a shaker water bath (see Table 1). Shaking speed was 150 rpm.

All sample group and control groups extracts were storage at ambient temperature after extracting. Extracts were used freshly within 8 h according to NEWAY SOP.

Table 1 Test Sample Extraction and Controls Preparation

GROUPs		Sampling Method	Sterilization Method	Extracting Ratio*	Extracting Condition	Extract Appearance
Test Sample	Conductive Hydrogel	Random of Hydrogel	Ultraviolet Irradiation (1h)	3cm^2:1ml	37℃, 24h	Transparent
Negative	HDPE	Random	Autoclave (121℃,20min)	0.2g:1ml	37℃, 24h	Transparent
Blank	MEM (with 10% FBS)	/	/	/	37℃, 24h	Transparent
Positive	5g/L phenol solution	/	/	/	37℃, 24h	Transparent

2. EXPERIMENTAL DESIGN

2.1 Experimental System

2.1.1 Cell Strain: NCTC clone 929 (L929)

2.1.2 Cell Source: Cell Center of Chinese Academy of Sciences

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

2.1.4 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.

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

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

2.1.7 Compliance: All activities of this study are carried out in compliance with the GLP (Good Laboratory Practices) for U.S. Food and Drug Administration Good Laboratory Practice Regulations, 21 CFR Part 58 (1987).

2.2 Test Procedure

2.2.1 Cell cultures are removed from culture flasks by enzymatic digestion (trypsin/EDTA) and the cell suspension is centrifuged (200*g*, 3min). The cells are then resuspended in culture medium and the cell suspension is adjusted at a density of 1×10^5 cells/ml. Using a multichannel pipette, dispense 100µl culture medium only (blank) into the peripheral wells of a 96-well tissue culture microtitre plate. Blank control, negative control, positive control and test sample extract (100%, 75%, 50%, 25%) groups are assigned to the remaining wells, dispense 100µl of a cell suspension of 1×10^5 cells/ml (= 1×10^4 cells/well).

2.2.2 Incubate cells for 24h (5% CO_2, 37°C, >90% humidity), examine each plate under a phase contrast microscope and record cell morphology, and then aspirate culture medium from the cells.

2.2.3 Per well, add 100µl of treatment medium containing either the appropriate concentration of sample extract (100%, 75%, 50%, 25%), or the negative control (100%), or the PC (100%),

or nothing but blank.

2.2.4 Incubate cells for 24h (5% CO_2, 37°C, > 90% humidity).

2.2.5 After 24 h treatment, examine each plate under a phase contrast microscope to identify systematic cell seeding errors and growth characteristics of control and treated cells. Record changes in the morphology of the cells due to cytotoxic effects of the test sample extract.

2.2.6 After the examination of the plates, remove the culture medium from the plates. 50µl of the MTT solution is then added to each test well and the plates are further incubated for 2h in the incubator at 37°C. Then the MTT solution is discanted and 100µl of isopropanol are added in each well. Sway this plate and subsequently transfer it to a microplate reader equipped with a 570nm filter to read the absorbance (reference wavelength 650nm).

2.2.7 All processes are completed in sterile environment with aseptic technique.

2.3 Data Analysis

2.3.1 Calculate the average value and standard deviation (SD) of optical density for each group.

2.3.2 Calculate the viability with below equation:

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

where

*OD*570e is the mean value of the measured optical density of the 100% extracts of the test sample;

*OD*570b is the mean value of the measured optical density of the blanks.

2.4 Evaluation Criteria

2.4.1 If viability is reduced to < 70% of the blank, it has a cytotoxic potential.

2.4.2 The viability result of test sample extract (100%) is used as the final reference.

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

3. TEST RESULTS

3.1 Cell Morphology

As shown in Table 2, the cells exposed to negative control showed no significant change in cell morphology compared to that of reagent control. Positive control extract caused severe cellular damage and obvious morphological alteration in almost all cells. The cells treated by

test sample extract showed no morphological change.

Table 2 Cell Morphology results

Group	Before seeding into 96-well plate	Before treatment with extract or control	After treated with extract or control for 24h
Positive Control	○	○	×
Negative Control		○	○
Blank Control		○	○
100% Extract		○	△
75% Extract		○	□
50% Extract		○	□
25% Extract		○	□

Remarks:

"○" Means none reactivity, discrete intracytoplasmatic granules, no cell lysis, no reduction of cell growth

"□" Means slight reactivity. 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.

"△" Means mild reactivity, Not more than 50% of the cells are round, devoid of intracytoplasmatic granules, no extensive cell lysis; not more than 50% growth inhibition observable.

"◇" Means moderate reactivity. Not more than 70% of the cell layers contain rounded cells or are lysed; cell layers not completely destroyed, but more than 50% growth inhibition observable.

"×" Means severe reactivity. Nearly complete or complete destruction of the cell layers.

3.2 Inhibition of cell viability

The results of this study are as below:

a) The Viab.% value of test sample extract (100%) is 73.16% (See Attachment I).

b) No cytotoxicity is detected in Negative Control group, meanwhile the cytotoxicity of Positive Control group is detected;

c) The mean OD_{570} value of blanks shall be ≥ 0.2, measured value is: 0.5172

d) Blanks are placed both at the left side (row 2) and the right side (row 11) of the 96-well plate, the left and the right mean of the blanks do not differ by more than 15% from the mean of all

blanks measured value is: 4.72%.

3.3 The test results apply to the tested sample in this study only. NEWAY is not responsible for further evaluation of these results. It is the responsibility of the sponsor to decide if this data is applicable to other samples.

4. DEVIATION

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

5. CONCLUSION

Under the conditions of this study, the cell viability of the test sample is 73.16%. Therefore, Test Sample induced non-cytotoxicity to L929 cells.

6. 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

All the records and test samples were handled according to GLP Guidance. Agent authorized by the sponsor can apply for the review according to NEWAY policy.

Attachment I MTT data

Group	OD 1 (570nm)	OD 2 (650nm)	OD_{570}	OD_{570} (Avg)	OD_{570} (SD)	OD_{570} (CV%)	Viab.%
Left Blank	0.633	0.097	0.5360	0.5416	0.02148	3.97	/
	0.654	0.113	0.5410				
	0.620	0.106	0.5140				
	0.646	0.103	0.5430				
	0.734	0.160	0.5740				
Negative	0.696	0.152	0.5440	0.5326	0.01250	2.35	102.98
	0.691	0.159	0.5320				
	0.647	0.132	0.5150				
	0.629	0.102	0.5270				
	0.647	0.102	0.5450				
25% **extract**	0.650	0.141	0.5090	0.4876	0.01881	3.86	94.28
	0.570	0.108	0.4620				
	0.573	0.098	0.4750				
	0.638	0.143	0.4950				
	0.595	0.098	0.4970				
50% **extract**	0.617	0.121	0.4960	0.4966	0.01311	2.64	96.02
	0.596	0.113	0.4830				
	0.642	0.124	0.5180				
	0.621	0.125	0.4960				
	0.591	0.101	0.4900				
75% **extract**	0.577	0.107	0.4700	0.4700	0.01170	2.49	90.87
	0.569	0.102	0.4670				
	0.654	0.201	0.4530				
	0.574	0.099	0.4750				
	0.598	0.113	0.4850				
100% extract	0.491	0.111	0.3800	0.3784	0.01864	4.92	**73.16**
	0.510	0.142	0.3680				
	0.496	0.116	0.3800				
	0.457	0.100	0.3570				
	0.496	0.089	0.4070				
Positive	0.056	0.056	0.0000	0.0068	0.00536	78.78	1.31
	0.059	0.052	0.0070				
	0.054	0.051	0.0030				
	0.142	0.130	0.0120				
	0.067	0.055	0.0120				
Right Blank	0.591	0.109	0.4820	0.4928	0.01489	3.02	/
	0.609	0.111	0.4980				
	0.638	0.122	0.5160				
	0.614	0.125	0.4890				
	0.571	0.092	0.4790				

blank below

广东纽唯质量技术服务有限公司

NEWAY TESTING LABORATORY

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