证书编号：2013100106S

TESTING
CNAS L2954

Sanitation & Environment Technology Institute, Soochow University, Final Report

Report Number: SDWH-M201403678

In Vitro Cytotoxicity Test of Electrode Gel G607
using ISO 10993-5:2009 Test Method
MTT Method
MEM with 10%FBS extract

Sponsor

GMDASZ Manufacturing Co.,Ltd.

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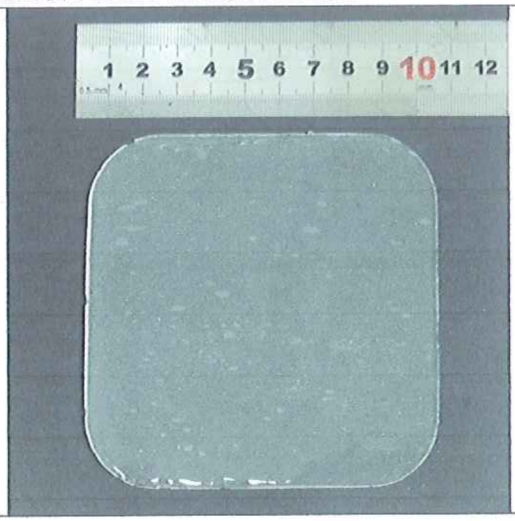
CONTENTS

SUPPLEMENTARY EXPLANATION

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 result relate only to the articles tested.
5. The report shall not be reproduced except in full without the written approval of the institute.

STUDY VERIFICATION AND SIGNATURE

Test Article	
Test Article Receipt	2014-11-11
Protocol No	SDWH-PROTOCOL-GLP-M201403678
Protocol Effective Date	2014-11-14
Technical Initiation Date	2014-11-17
Technical Completion Date	2014-11-19
Final Report Completion Date	2014-12-08

Edited by : Mei Chao 2014-12-08
Date

Checked by : 2014-12-09
Study Director
Date

Approved by : 2014-12-10
Authorized signatory
Date

Sanitation & Environment Technology Institute, Soochow University

QUALITY ASSURANCE STATEMENT

This study was conducted in compliance with 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 SDWH, 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.

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

INSPECTIONS	DATE OF INSPECTION	DATE REPORTED STUDY DIRECTOR	DATE REPORTED MANAGEMENT
EXPERIMENTAL PROCEDURE	2014-11-17	2014-11-17	2014-11-17
RAW DATA	2014-12-08	2014-12-08	2014-12-08
AMENDMENT REPORT	2014-12-08	2014-12-08	2014-12-08

Quality Assurance Unit : Zhang Mochu

2014.12.8
Date

1.0 Study Summary

The testarticle extract was added to growth media containing L-929 cells in 96 well plates and then incubated at 37℃ in 5% CO_2 for 24h to determine the potential cytotoxicity. The MTT method results showed that the cytotoxicity ratio of the 100 % test article extract was 94.1% and the results of control groups showed the test was valid.
Under the conditions of this study, the test article Electrode Gel G607 extract did not show potential toxicity to L-929 cells.

2.0 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.

3.0 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)

4.0 Compliance

Good Laboratory Practice Regulations, 21 CFR, Part 58
ISO/IEC17025:2005 General requirements for the competence of testing and calibration laboratories (CNAS-CL01 Accreditation Criteria for the competence of testing and calibration laboratories)
China National Accreditation Service for Conformity Assessment Laboratory Accreditation Certificate No.CNAS L2954

Accreditation Criteria for the competence of the laboratories (Quality and Technical Bureau of Jiangsu Province Metrology Accreditation Certificate CMA 2013100106S)

5.0 Identification of test and control articles

5.1 Test article name: Electrode Gel G607
Test article initial state: Not Sterilized
CAS/Code#: Not Supplied by Sponsor (N/S)
Size: 10×10cm
Lot/ Batch#: 20141107
Physical State: Solid
Color: Colorless
Density: 1.1g/cm^3
Stability: Stable
Solubility: N/S

Storage Condition: N/S
Test article material: Hydrogel
Packaging Material: PE Bag
The information about the test article was supplied by the sponsor wherever applicable.
The Sponsor was responsible for all test article characterization data as specified in the GLP Regulations.
Extracting solvent: MEM medium, with addition 10% FBS

5.2 Negative Control Article Name: High Density Polyethylene
Manufacturer: U.S.Pharmacopeia
Size: 3 Strips
Lot/Batch#: J0L476
Physical State: Solid
Color: White
Stability: Stable at room temperature
Storage Conditions: Room temperature
Extracting solvent: MEM medium, with addition 10% FBS

5.3 Positive Control Article Name: Phenol
Manufacturer: Sinopharm Chemical Reagent Co.,Ltd
Size: 500g
Lot/Batch#: T20110909
Concentration: 0.5%
Solvent: MEM medium, with addition 10% FBS
Date prepared: 2014-11-17
Physical State: Liquid
Color: Pink
Storage Condition: 4 ± 2 ℃

5.4 Blank Control Name: MEM medium, with addition 10% FBS
Date prepared: 2014-11-17
Physical State: Liquid
Color: Pink
Storage Condition: 4 ± 2 ℃

6.0 Identification of test system

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

7.0 Justification of the test system

Historically, mouse fibroblast L929 cells have been used for cytotoxicity studies because they demonstrate sensitivity to extractable cytotoxic articles.

8.0 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.

9.0 Experiment design

9.1 Sample and Control Preparation

Aseptic extracting the test article (test article to volume of vehicle) by MEM medium(10%FBS) according to the table below .Sealed and incubated at 37℃ and 5% CO_2 for 24h.Extracts were be used immediately after extraction.

Aseptic Sampling			Sterilization	Aseptic Extraction In Inert Container		Final Extract	
Ratio	Sampling Manner	Actually sampling	Method	Extracts	Condition	pH	Clear or Not
$3cm^2$: 1ml	Random sampling (Remove the protective films) Perform the extraction; add additional volume of extraction vehicle that the test article absorbs (12.5ml/g, provided by sponsor).	$60cm^2$	Cobalt- 60 25kGy	193ml	37℃,24h	7.4	Clear

The blank control (vehicle), negative and positive controls were similarly prepared.

9.2 Equipment

Autoclaves (SDWH-030),Calibration Expire(2015-05-28),
CO_2 Incubator (SDWH-021),Calibration Expire(2015-10-22),
CO_2 Incubator (SDWH-186),Calibration Expire(2015-09-12),
Inverted microscope (SDWH-037),
Steel Straight Scale (SDWH-463),Calibration Expire(2015-10-14),
Electronic Balance (SDWH-056),Calibration Expire(2015-03-20),
Clean bench (SDWH-454),Calibration Expire(2015-10-26),
Power Wave XS Microplate Reader (SDWH-312),Calibration Expire(2015-10-11).

9.3 Reagents

MTT
(3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyletrazolium bromide)(SIGMA ,Lot No: MKBR6576V)
FBS (GiBco , Lot No: 1428479)
Trypsinase (GiBco , Lot No: 1516530)
Penicillin, Streptomycin sulfate (GiBco, Lot No: 1430661)
MEM (GiBco , Lot No: L40112)
99.9%Isopropanol (Sinopharm Chemical Reagent Co.,Ltd , Lot No:20130328).

9.4 Test Method

Aseptic procedures were used handling of cell cultures.

L929 cells were cultured in MEM medium (10% FBS, Penicillin 100 U/ml, Streptomycin sulfate 100 μg/ml) at 37℃ in a humidified atmosphere of 5% CO_2, then digested by 0.5% trypsin containing EDTA to get single cell suspension. And obtain a 1×10^5 cells/ml suspension by centrifuging (200g,3min) and re-dispersing in MEM medium finally.

The suspended cells were dispensed at 100μl per well in 96-well plate, and culture it in cell incubator (5% CO_2, 37℃, >90%humidity), 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 of extract of test article (100%、75%、50%、25%), control article, negative article (100%) and positive article (100%) respectively. Incubate the 96-well plate at 37℃ in cell incubator of 5% CO_2 for 24 h. Five replicates of each test were tested.

After 24h incubation, observe the cell morphology first and then discard the culture medium. A 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 2 hours. The liquid in each well was tipped out and 100 μl 99.9% isopropanol 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 and reference wavelength at 650 nm.

9.5 Results of Cell Morphology

Table 1 Observation of the 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.

9.6 Results of the Cell Vitality

Table2 Results of the Cell Vitality

Group	$\bar{x}\pm s$	Viability%
Blank control	0.367±0.020	100.0%
Negative control	0.368±0.015	100.2%
Positive control	0.013±0.001	3.4%
100% test article extract	0.346±0.008	94.1%
75% test article extract	0.351±0.011	95.6%
50% test article extract	0.358±0.015	97.5%
25% test article extract	0.364±0.006	99.2%

9.7 Statistical Method

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

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%.

9.8 Evaluation Criteria

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.

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

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

The Viab.% of the 100% extract of the test article is the final result.

9.9 Conclusion

Under the conditions of this study, the test article Electrode Gel G607 extract did not show potential toxicity to L-929 cells.

10.0 Record Storage

All raw data pertaining to this study and a copy of the final report are retained in designated SDWH archive.

11.0 Confidentiality Agreement

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