GLP Final Report

Report No.: T51642018-001(E)

Exclusively prepared for:

SPONSOR

Solaplus biotech co., ltd.
No.75 FengFang Road, Ouhai Economic Development Zone, Wenzhou

STUDY TITLE

Cytotoxicity Study Using Direct Contact Method

TEST ARTICLE

Hemostatic Xerogel Sponge
Model: XLJ-I

ANAB ACCREDITED ISO/IEC 17025 TESTING LABORATORY
AT-2046

ilac-MRA

TESTING FACILITY:
Mid-Link Technology Testing Co., Ltd.
B6-05, RongTong Building,
No. 80, Haiyun Street, TEDA
Tianjin, 300457, China

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Summary

The test article, Hemostatic Xerogel Sponge, XLJ-I, was placed on the cell surface to evaluate the cytotoxic properties with growing-well L-929 cell, after incubating at 37°C in 5% CO_2 for 24hours. Decolorization zone around the test and controls articles using an inverted microscope with a calibrated screen was assessed and reactivity for each article determined in accordance with pre-determined criteria.

Under the conditions of this study, results showed that reactivity grades of test article were 2. The test article would be considered non-cytotoxic. The negative controls and the positive controls performed as anticipated.

Approved by: _____ 12/27/2018

Shixia Wang, Study Director — Date

GLP STATEMENT

This nonclinical laboratory study was conducted in accordance with the United States Food and Drug Administration Good Laboratory Practice Regulations, 21 CFR Part 58.

There was no deviation to the protocol or provisions of GLP Regulation noted during the course of the study.

Approved by: _____ 12/27/2018

Shixia Wang, Study Director — Date

1. Generals

1.1 Purpose

The purpose of this study was to evaluate the cytotoxicity of the test article to L-929 cell.

1.2 Guidelines

This study will be conducted based on the requirements of

(1) ISO 10993-1, Biological evaluation of medical devices - Part 1: Evaluation and testing within a risk management process (2018).

(2) ISO 10993-5, Biological evaluation of medical devices - Part 5: Tests for in vitro cytotoxicity (2009).

1.3 Compliance

This nonclinical laboratory study will be conducted in accordance with:

(1) International Organization for Standardization (ISO) 17025 - General requirements for the competence of testing and calibration laboratories (2005);

(2) The United States Food and Drug Administration Good Laboratory Practice Regulations, 21 CFR Part 58.

1.4 Dates

Test Article Received:	11/07/2018
Initiated:	11/26/2018
Observations Concluded:	11/28/2018

2. Materials

Test Article	Hemostatic Xerogel Sponge
Model	XLJ-I
Manufacturer	Same as sponsor
Identification Number	Not provided
Status	Sterile
Physical Description	White, Flake sponge, Solid
Composition	Chitosan, Sodium polyacrylate, Polyethylene glycol
Stability	Stability was determined by and on file with the sponsor.
Expiration Date (or Shelf Life)	Two years
Strength	Not applicable, no active ingredient
Purity	Not applicable, no active ingredient
Storage Condition	Room Temperature
Note	Information regarding the test article characterization was provided by sponsor in the Sample Submission Form.
Negative Control Article	High density polyethylene (HDPE)
Manufacturer	Kunshan Fei Yao Plastic Products Co., Ltd.
Lot Number	20180619
Stability	Marketed product, stability is characterized by its labelling
Composition, Strength, Purity or other characteristics	Not applicable, no active ingredient

Storage Condition	Room Temperature
Positive Control Article	Latex gloves
Manufacturer	TG MEDICAL SDN. BHD.
Lot Number	5112004347
Stability	Marketed product, stability is characterized by its labelling
Composition, Strength, Purity or other characteristics	Natural rubber latex
Storage Condition	Room Temperature
Medium	Minimum Essential Medium (MEM)
Manufacturer	Sigma-Aldrich. Inc
Lot Number	SLBV3954
Stability	Stable during the study.
Composition, Strength, Purity or other characteristics:	Minimum Essential Medium (MEM) supplemented with 10% fetal bovine serum, 100IU/ml Penicillin G and 100μg/ml streptomycin
Storage Condition	2-8°C
Reagent	1% Neutral Red
Manufacturer	Beijing Solarbio Science & Technology Co., Ltd.
Lot Number	20161224
Preparation	The test article, negative control article and positive control article were cut into a circular sample with diameter of 11 mm, with a flat surface to ensure adequate contact with the cell overlay.

3. Test Systems

3.1 Test System and Justification

Mammalian cell culture monolayer consisting of L-929 mouse fibroblast cells will be used. In vitro mammalian cell culture studies have been used historically to evaluate cytotoxicity of biomaterials and medical devices. All stock cultures of cells will be tested to confirm the absence of mycoplasma contamination.

3.2 Test System Management

L-929 mouse fibroblast cells will be propagated and maintained in culture flasks containing MEM at (37 ± 1)°C with 5% carbon dioxide (CO_2). For this study, 25 cm2 culture flasks will be seeded, labeled with passage number and date, and incubated at (37 ± 1)°C in 5% CO2 to obtain subconfluent monolayers of cells prior to use. Aseptic procedures will be used in the handling of the cell cultures following approved MID-LINK Standard Operating Procedures.

4. Method

The cells are cultured in MEM medium at (37 ± 1)°C in 5% CO_2 to reach the end of the log growth phase, and then suspended with MEM medium to obtain cell suspension with concentration of 2.5×10^5 cells/ml. Pipette the 2 ml of cell suspension into 3.5 cm Petri dishes and incubate at (37 ± 1) °C in a water-saturated atmosphere with 5 % (volume fraction) CO_2 for 24 h. After incubate for 24 h, verify the subconfluency and the morphology of the cultures

with a microscope before starting the test. Remove and discard the culture medium. Carefully place individual specimens of the test sample on the cell layer in the center of each of the replicate Petri dishes. Ensure that the specimen covers approximately one tenth of the cell layer surface. Exercise care to prevent unnecessary movement of the specimens, as this could cause physical trauma to the cells. For example, patches of dislodged cells can result from unnecessary movement. Then add fresh 2 ml culture medium to each 3.5 cm Petri dishes. Incubate at (37 ± 1) °C in a water-saturated atmosphere with 5 % (volume fraction) CO_2 for 24 h. Triplicate sample are prepared .

Prepare replicate Petri dishes for both the negative control and positive control material. Incubate the Petri dishes under the same conditions for 24 h corresponding to the selected specific assay.

Discard the supernatant culture medium before adding chemicals/dyes. Add 2 ml neutral red solution and keep dark for 15 min to 20 min. Aspirate excess neutral red solution. Protect the culture from light in the presence of neutral red, as the cells can be damaged.

5. Evaluation and Statistical Analysis

Assess the decolorization zone around the test and controls samples using an inverted microscope with a calibrated screen, and determine reactivity for each sample in accordance with the criteria specified in Table 1

Table 1 Reactivity grades for direct contact test

Grade	Reactivity	Description of reactivity zone
0	None	No detectable decolorization 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

The achievement of a numerical grade greater than 2, based on Tables 1, is considered a cytotoxic effect.

6. Results

See Attachment 2: Results

7. Conclusion

Under the conditions of this study, results showed that reactivity grades of test article were 2. The test article would be considered no cytotoxicity potential. The negative controls and the positive controls performed as anticipated.

Results and conclusions apply only to the test article tested. Any extrapolation of these data to other articles is the sponsor's responsibility.

8. Records

All raw data pertaining to this study and a copy of final report will be retained in designated Mid-Link's archive files in accordance with Mid-Link SOP.

STATEMENT OF QUALITY ASSURANCE ACTIVITIES

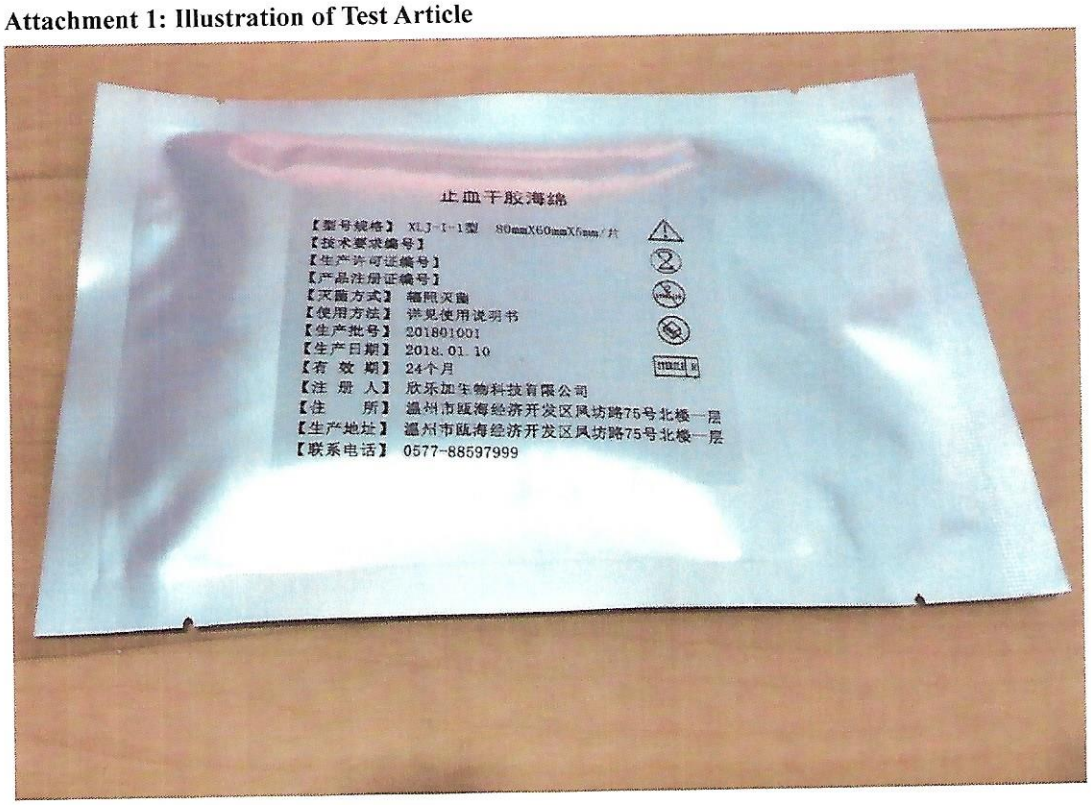
Phase Inspected	Date Inspected
Dosing	11/27/2018
Study Data Review	11/28/2018
Final Report Review	12/07/2018

Based on a review of this study, it has been concluded that this report accurately describes the methods and standard operating procedures, and that the reported results accurately reflect the raw data of the study. This study has been reviewed in accordance with the provisions of the FDA Good Laboratory Practice Regulations (21 CFR, part 58).

QA Representative 刘婷慧 12/27/2018

Authorized Signature Date

Attachment 1: Illustration of Test Article



Attachment 2: Results

Table A1 Observation of the cell morphology

group	Before treatment	24h after treatment
Test article	Discrete intracytoplasmatic granules, no cell lysis, no reduction of cell growth	Zone limited to area under specimen, the cells were round , devoid of intracytoplasmatic granules, but some cells growth inhibition observable.
Negative control	Discrete intracytoplasmatic granules, no cell lysis, no reduction of cell growth	Discrete intracytoplasmatic granules, no cell lysis, no reduction of cell growth
Positive control	Discrete intracytoplasmatic granules, no cell lysis, no reduction of cell growth	Zone extending farther than 1,0 cm beyond specimen. The cell layers contain rounded cells or are lysed. Nearly complete or complete destruction of the cell layers.

Table A2 Results

Group		Grade	Reactivity
Test article	Petri dish #1	2	Mild
	Petri dish #2	2	Mild
	Petri dish #3	2	Mild
Negative control	Petri dish #1	0	None
	Petri dish #2	0	None
	Petri dish #3	0	None
Positive control	Petri dish #1	4	Severe
	Petri dish #2	4	Severe
	Petri dish #3	4	Severe