GLP Final Report

Report No.: T60122018-002(E)
Date: 05/07/2018

Exclusively prepared for:

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

STUDY TITLE
ISO Guinea Pig Maximization Sensitization Test

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

Table of Content

Summary

The test article, hemostatic xerogel sponge , XLJ-Ⅰ, was evaluated for the potential to cause delayed dermal contact sensitization in a guinea pig maximization test. This study was conducted based on the requirements of ISO 10993-10, Biological evaluation of medical devices - Part 10: Tests for irritation and skin sensitization.

The test article was extracted in polar extract (0.9% sodium chloride (SC)) and non-polar extract (Cotton Seed Oil (CSO)). Each extract was intradermally injected and occlusively patched to ten test guinea pigs (per extract). The extraction vehicles were similarly injected and occlusively patched to five control guinea pigs (per vehicle). Following a recovery period, the test and control animals received a challenge patch of the appropriate test article extract and the vehicle control. All sites were scored for dermal reactions at 24 and 48 hours after patch removal.

The test article extracts showed no evidence of causing delayed dermal contact sensitization in the guinea pig. The test article was not considered a sensitizer in the guinea pig maximization test.

Approved by: Xiaojie Bo 05/07/2018

Xiaojie Bo, 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: Xiaojie Bo
Xiaojie Bo, Study Director

05/07/2018
Date

1. Generals

1.1 Purpose

The purpose of this study was to evaluate the potential of the test article to cause delayed dermal contact sensitization in the guinea pig maximization test.

1.2 Guidelines

This study was conducted based on the International Organization for Standardization 10993-10, Biological evaluation of medical devices – Part 10: Tests for irritation and skin sensitization.

1.3 Dates

Test Article Received:	03/12/2018
Intradermal Induction Started:	04/09/2018
Observations Concluded:	05/05/2018

2. Materials

Test Article	hemostatic xerogel sponge
Model	XLJ-I
Status	Sterile Finished Device Gamma Radiation Sterilization
Physical Description	White, Flaky sponge, Solid
Composition	Chitosan, Sodium polyacrylate, Polyethylene glycol
Stability	Stability testing is completed and on file with the sponsor Expiration Date: 2 years
Strength	Not applicable, no active ingredient
Purity	Not applicable, no active ingredient
Storage Condition	Room Temperature

Extraction Vehicle (Control) Polar	0.9% sodium chloride
Manufacturer	China Otsuka Pharmaceutical Co.,Ltd.
Lot Number	7K70G3
Physical Description	Clear, Colourless, Liquid
Composition	NaCl
Strength	500ml：4.5g
Purity	Conforms to China Pharmacopeia (2015)
Stability	Marketed product, stability is characterized by its labelling
Storage Condition	Room Temperature

Extraction Vehicle (Control) Non-Polar	Cotton Seed oil
Manufacturer	Acros Organics
Lot Number	A0387833
Physical Description	Clear, Yellow to Green, Liquid

Composition	Cotton Seed Oil
Strength	500mL
Purity	Pure
Stability	Marketed product, stability is characterized by its labelling
Storage Condition	Room Temperature

Reagent	FREUND'S COMPLETE ADJUVANT
Manufacturer	MP Biomedical LLC
Lot Number	07414
Physical Description	Emulsion
Composition	Mycobacterium, Arlacel A and paraffin oil
Strength	10ml
Purity	25 mg of Mycobacterium, 7.5 mL Arlacel A and 42.5 mL paraffin oil.
Stability	Marketed product, stability is characterized by its labelling
Storage Condition	2-8℃

Reagent	Sodium Dodecyl Sulfate (SDS)
Manufacturer	Tianjin OKLABS Commercial and Trading Co., Ltd.
Lot Number	20151011
Physical Description	White, dry and crystal
Composition	$CH_3(CH_2)OSO_3Na$
Stability	Marketed product, stability was characterized by labeling
Specification	500g
Storage Condition	Sealed dry preservation

Reagent	Medical vaseline
Manufacturer	ShanDong LIRCON Medical Technology Incorporated Company
Lot Number	171201
Physical Description	White semi-liquid mixture
Stability	Marketed product, stability was characterized by labeling
Specification	500ml
Storage Condition	Sealed preservation

Extractions Procedure

The sample was saturated in the extraction medium before extraction.The test article and the control blank (extraction vehicle without the test article) were subjected to the extraction conditions as described below. The extracts were continuously agitated during extraction.

Extraction:	**Polar (SC)**		**Non-Polar (CSO)**	
Intradermal Induction	**Test**	**Control**	**Test**	**Control**
Extraction Ratio	0.1g: 1ml	/	0.1g: 1ml	/
Sample Amount	2.26 g	/	2.14 g	/
Extraction Vehicle Volume	22.6 ml	20.0 ml	21.4 ml	20.0 ml

Extraction Condition	50℃ 72 hour	50℃ 72 hour	50℃ 72 hour	50℃ 72 hour
Condition of Extracts	Clear	Clear	Clear	Clear
	No Particulate	No Particulate	No Particulate	No Particulate

Extraction:	**Polar (SC)**		**Non-Polar (CSO)**	
Topical Induction	**Test**	**Control**	**Test**	**Control**
Extraction Ratio	0.1g: 1ml	/	0.1g: 1ml	/
Sample Amount	3.50 g	/	3.50 g	/
Extraction Vehicle Volume	35.0 ml	20.0 ml	35.0 ml	20.0 ml
Extraction Condition	50℃ 72 hour	50℃ 72 hour	50℃ 72 hour	50℃ 72 hour
Condition of Extracts	Clear	Clear	Clear	Clear
	No Particulate	No Particulate	No Particulate	No Particulate

Extraction:	**Polar (SC)**		**Non-Polar (CSO)**	
Challenge	**Test**	**Control**	**Test**	**Control**
Extraction Ratio	0.1g: 1ml	/	0.1g: 1ml	/
Sample Amount	3.10 g	/	3.30 g	/
Extraction Vehicle Volume	31.0 ml	20.0 ml	33.0 ml	20.0 ml
Extraction Condition	50℃ 72 hour	50℃ 72 hour	50℃ 72 hour	50℃ 72 hour
Condition of Extracts	Clear	Clear	Clear	Clear
	No Particulate	No Particulate	No Particulate	No Particulate

Note: All extracts were not centrifuged, filtered or otherwise altered prior to dosing. It was dosed immediately after extraction.

3. Test Systems and Justification

Species:	Guinea pig (Caviaporcellus)
Breed:	Hartley
Source:	Tianjin Yuda Laboratory Animal Breeding Co., Ltd.
Sex:	Male and Female females were nulliparous and non-pregnant
Body Weight Range:	300-500 grams at study initiation
Age:	Young adults
Acclimation Period:	Minimum 5 days
Number of Animals:	Thirty (30)
	10 SC Test Group, 5 SC Control Group and 10 CSO Test Group, 5 CSO Control Group
Identification Method:	Ear tag

Justification: The Hartley albino guinea pig (animal) has been used historically for sensitization studies (Magnusson and Kligman, 1970). The guinea pig is believed to be the most sensitive animal model for this type of study. The susceptibility of the Hartley strain to a known sensitizing agent, 1-chloro-2,4-dinitrobenzene (DNCB) has been substantiated at MID-LINK with this method. Detail information is provided in ATTACHMENT: Positive Study Record.

4. Animal Management

4.1 Husbandry, Housing and Environment

Conditions conform to MID-LINK Standard Operating Procedures that are based on the "Guide for the Care and Use of Laboratory Animals." Animals will be individually housed in stainless steel or plastic suspended cages identified by a card indicating the animal number, test identification, sex, and date dosed.

4.2 Food, Water and Contaminants

A commercially available guinea pig feed was provided daily. Potable water was provided ad libitum through species appropriate water containers or delivered through an automatic watering system. No contaminant present in the feed and water was expected to impact the results of this study.

4.3 Personnel

Associates involved in this study were appropriately qualified and trained.

4.4 Sedation, Analgesia or Anesthesia

It has been determined that the use of sedation, analgesia or anesthesia was not necessary during the routine course of this procedure.

4.5 Veterinary Care

All anesthetics, analgesics, and other medications are given or altered at the discretion of the attending veterinarian in accordance with standard veterinary practice and the study objectives. This applies to specific medication, dose, and dosing intervals. In the unlikely event that an animal should become injured, ill, or moribund, care was conducted in accordance with current veterinary medical practice. If warranted for humane reasons, euthanasia will be conducted in accordance with the current report of the American Veterinary Medical Association's Guidelines on Euthanasia. The objective of the study is given due consideration in any decision and the study sponsor will be advised.

4.6 Selection

Only healthy, thin-skinned animals free of mechanical irritation or trauma that could interfere with the test were selected.

5. Methods

On the first day of treatment, fifteen (15) animals per extract (ten tests, five controls) were weighed. The fur from the dorsoscapular area of the animals was removed with an electric clipper.

5.1 Intradermal Induction

Three pair of intradermal injections was administered to the animals within an approximate 2 cm × 4 cm area over the dorsoscapular region as follows:

Test Animals

a 0.1 mL of 50:50 (v/v) mixture of FCA and the chosen vehicle

b 0.1 mL of test extract

c 0.1ml of a 1:1 mixture of the 50：50（V/V）vehicle/FCA mixture and the test extract

Control Animals

a 0.1 mL of 50:50 (v/v) mixture of FCA and the chosen vehicle

b 0.1 mL of vehicle

c 0.1 mL of a 1:1 mixture of the 50:50 (v/v) FCA and the vehicle

5.2 Topical Induction

At 6 days after completion of the Intradermal Induction injection, the injection sites were clipped free of fur again and treated with a 10% (w/w) sodium dodecyl sulfate (SDS). The SDS suspension was applied in an amount sufficient to coat the skin unless the animal exhibit excessive redness and/or swelling at site b.

After 24 hours any remaining SDS residue was gently wiped from the area with gauze. Following removal of

the SDS, an approximate 2 cm × 4 cm filter paper patch, saturated with approximately 0.5mL of the test extract preparation (test animals) or vehicle (control animals) was applied over the same injection area and secured with a nonreactive tape. The trunk of each animal was then wrapped snugly with an elastic band for 48 hours.

5.3 Challenge

At 14 days after unwrapping Topical Induction wraps, the fur was clipped from the sides and flanks with an electric clipper. Two nonwoven cotton disks backed by a flexible chamber and semi-occlusive hypoallergenic tape were saturated with approximately 0.5 mL of freshly prepared test extract and blank vehicle, respectively and applied to the left and right flank or dorsum of each animal, respectively. The trunk of each animal was wrapped to maintain well-occluded sites. At 24 hours, the wraps and patches were removed and any residue remaining at the sites will be wiped with gauze.

5.4 Laboratory Observations

a) Animals were observed daily for general health.

b) Body weights were recorded at pretreatment.

c) Observations for dermal reactions were conducted at 24 and 48 hours after patch removal. If necessary, the sites will be wiped with 35% isopropyl alcohol and/or the fur will be clipped to facilitate scoring. Dermal reactions will be scored in accordance with the criteria shown below:

Table 1: Grading Scale

Patch Test Reaction	Grading Scale
No visible change	0
Discrete or patchy erythema	1
Moderate and confluent erythema	2
Intense erythema and swelling	3

6. Evaluation

The responses from the challenge phase were compared within the test animal group and between test and control conditions. In the final analysis of data, consideration was given to the overall pattern, intensity, duration and character of reactions of the test as compared to the control conditions. The control conditions are (1) the control vehicle on the test animals, (2) the test on the control animals, and (3) the control vehicle on the control animals. Statistical manipulation of data was not applicable to this study. Grades of 1 or greater observed in the test group generally indicated sensitization, provided that grades of less than 1 were observed on the control animals. If grades of 1 or greater were noted on control animals, then the reactions of test animals that exceeded the most severe control reaction were considered to be due to sensitization.

7. Results

7.1 Clinical Observations and Body Weight Data

All animals were clinically normal throughout the study. The clinical observations and individual body weights at pretreatment are presented in ATTACHMENT.

7.2 Dermal Observations

No evidence of sensitization was observed. Individual results of dermal scoring for the challenge phase are presented in ATTACHMENT.

8. Conclusion

Under the conditions of this study, the test article extracts showed no evidence of causing delayed dermal contact

sensitization in the guinea pig. The test article was not considered a sensitizer in the guinea pig maximization test.

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

9. Records

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

10. References

1. Code of Federal Regulations (CFR), Title 21, Part 58, Good Laboratory Practice for Nonclinical Laboratory Studies.
2. International Organization for Standardization (ISO) 17025 - General requirements for the competence of testing and calibration laboratories (2005)
3. International Organization for Standardization (ISO) 10993-1, Biological evaluation of medical devices - Part 1: Evaluation and testing within a risk management process (2009).
4. International Organization for Standardization 10993-10, Biological evaluation of medical devices – Part 10: Tests for irritation and skin sensitization. (2010)
5. International Organization for Standardization (ISO) 10993-12, Biological evaluation of medical devices - Part 12: Sample preparation and reference materials (2012).
6. GLP Study Protocol, T60122018-002.

STATEMENT OF QUALITY ASSURANCE ACTIVITIES

Phase Inspected	Date Inspected	Date Reported to Study Director	Date Reported to Management
Observation(24h)	05/04/2018	05/04/2018	05/04/2018
Study Data Review	05/05/2018	05/05/2018	05/05/2018
Final Report Review	05/07/2018	05/07/2018	05/07/2018

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 李怀慧 Authorized Signature 2018.05.07 Date

ATTACHMENT: Clinical Observations and Individual Body Weight Data and Dermal Reaction

Treatment Group	Animal Number		Individual Observation		Dermal Reaction	
			Pretreatment Body Weight (g)	Clinical Observations	24 Hours	48 Hours
Test(SC)	1	A0240	449.2	No finding	0	0
	2	A0234	450.1	No finding	0	0
	3	A0232	424.6	No finding	0	0
	4	A0233	406.5	No finding	0	0
	5	A0238	467.3	No finding	0	0
	6	A0236	442.9	No finding	0	0
	7	A0239	457.6	No finding	0	0
	8	A0237	437.9	No finding	0	0
	9	A0231	446.8	No finding	0	0
	10	A0235	493.2	No finding	0	0
Control (SC)	11	A0227	445.2	No finding	0	0
	12	A0230	463.7	No finding	0	0
	13	A0228	459.5	No finding	0	0
	14	A0226	480.5	No finding	0	0
	15	A0229	470.8	No finding	0	0
Test (CSO)	16	A0224	432.4	No finding	0	0
	17	A0208	441.2	No finding	0	0
	18	A0222	474.7	No finding	0	0
	19	A0210	486.6	No finding	0	0
	20	A0206	495.3	No finding	0	0
	21	A0209	429.2	No finding	0	0
	22	A0225	441.1	No finding	0	0
	23	A0207	434.1	No finding	0	0
	24	A0223	411.4	No finding	0	0
	25	A0221	473.3	No finding	0	0
Control (CSO)	26	A0202	424.2	No finding	0	0
	27	A0204	479.6	No finding	0	0
	28	A0205	493.4	No finding	0	0
	29	A0203	431.3	No finding	0	0
	30	A0201	417.1	No finding	0	0

ATTACHMENT: ILLUSTRATION OF TEST ARTICLE

ATTACHMENT: Positive Control Study Record

What was tested 1-chloro-2,4-dinitrobenzene (DNCB)

Dates
Treatment Started: 01/09/2018
Observations Concluded: 02/04/2018

Purpose A periodic positive control study was conducted for the Guinea Pig Maximization Test to meet the following objectives: 1) confirm the methodology in ISO 10993-10, Biological Evaluation of Medical Devices - Part 10: Tests for Irritation and Skin Sensitization, 2) substantiate the potential of DNCB to cause delayed dermal contact sensitization and 3) substantiate the susceptibility of the Hartley guinea pig strain provided by to dermal contact sensitization.

Methods The test utilized young adult, nulliparous and not pregnant, female Hartley albino guinea pigs. The weight at study initiation ranged from 300 grams to 500 grams. A 0.1% (w/w) concentration of DNCB in ethanol absolute was intradermally injected and occlusively patched (7 days after injection) to ten test guinea pigs in an attempt to induce sensitization. Following a recovery period (14 days), the animals received a challenge patch of 0.1% (w/w) DNCB in ethanol absolute and ethanol absolute alone. Dermal reactions were scored at 24 and 48 hours after patch removal using the following scale:

Table 1: Grading Scale

Patch Test Reaction	Grading Scale
No visible change	0
Discrete or patchy erythema	1
Moderate and confluent erythema	2
Intense erythema and swelling	3

Table 2 Results

Anima Number	Dermal Reaction		Result
	24 Hours	48 Hours	
A0739	2	2	+
A0738	1	2	+
A0740	1	1	+
A0737	1	1	+
A0778	1	1	+
A0731	1	1	+
A0734	1	1	+
A0735	1	1	+
A0733	2	2	+
A0779	2	2	+

Conclusion Under the conditions of this study, the positive control substance showed evidence of causing delayed dermal contact sensitization in the guinea pig.