GLP Final Report

Report No.: T60122018-003(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 Intracutaneous Study in Rabbits

TEST ARTICLE
hemostatic xerogel sponge
Model: XLJ-Ⅰ

ANAB ACCREDITED ISO/IEC 17025 TESTING LABORATORY
AT-2046

ilac-MRA

TESTING FACILITY
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Table of Content

Summary

The test article, hemostatic xerogel sponge , XLJ- Ⅰ was evaluated for the potential to cause irritation following intracutaneous injection in rabbits. This study was conducted based on ISO 10993-10, Biological evaluation of medical devices – Part 10: Tests for irritation and skin sensitization.

The test article was extracted in polar extraction vehicle (0.9% sodium chloride solution (SC)) and non-polar extraction vehicle (Cotton Seed Oil (CSO)). Due to concern with the crowding and subsequent obscuring of injection sites, the test and control sites were cranial and caudal on the same side of the back as defined in the ISO standards.The injection sites were observed immediately after injection. Observations for erythema and edema were conducted at 24, 48 and 72 hours after injection.

Under the conditions of this study, the test article (polar extract group) met the requirements of the test since the difference between test extract overall mean score and corresponding control overall mean score was 0; the test article (non-polar extract group) met the requirements of the test since the difference between test extract overall mean score and corresponding control overall mean score was 0.

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 local dermal irritation of a test article extract following intracutaneous injection in rabbits.

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
Injection:	04/12/2018
Observations Concluded:	04/15/2018

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

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.

Group	**Polar (SC)**		**Non-Polar (CSO)**	
	Test	**Control**	**Test**	**Control**
Extraction Ratio	0.1g: 1ml	/	0.1g: 1ml	/
Sample Amount	3.4043 g	/	3.2816 g	/
Extraction Vehicle Volume	34.0 ml	20.0 ml	32.8 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:	Rabbit
Breed:	Japanese White
Source:	Tianjin Yuda Laboratory Animal Breeding Co., Ltd.
Body Weight Range:	2.00 kg above
Sex:	Male
Age:	Young adults
Acclimation Period:	Minimum 5 days
Number of Animals:	Three (3)
Identification Method:	Ear tag

Justification: The intracutaneous injection test in rabbits is specified in the current ISO testing standards and has been used historically to evaluate biomaterial extracts Method.

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 rabbit 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

Prior to treatment each animal was identified and weighed. Within a 4 to 18 hours period before treatment, each rabbit was clipped free of fur from the back and both sides of the spinal column to yield a sufficient injection area. If necessary, swab the skin lightly with 35% isopropyl alcohol (IPA) and allow to dry prior to injection. Due to concern with the crowding and subsequent obscuring of injection sites, the test and control sites were cranial and caudal on the same side of the back as defined in the ISO standards. A 0.2 mL dose of the appropriate test article extract was injected by the intracutaneous route into five separate sites on the right side of the back of each rabbit. Similarly, the corresponding control was injected on the left side of the back of each rabbit. No more than two test extracts and the corresponding controls were injected into each animal. Injections were about 2 cm apart. The appearance of the injection sites was noted immediately after injection. Observations for erythema and edema were noted for each injection site at 24, 48 and 72 hours after injection. Wipe the skin lightly with 35% IPA as necessary to facilitate scoring of the injection sites. Reactions were scored on a 0 to 4 basis. Other adverse changes at the injection sites were also noted. The reactions were evaluated according to the subjective rating scale as shown below:

Table 2: Test Scoring

Score	Erythema (ER)	Edema (ED)
0	No erythema	No edema
1	Very slight erythema (barely perceptible)	Very slight edema (barely perceptible)
2	Well-defined erythema	Well-defined edema (edges of area well-defined by definite raising)
3	Moderate erythema	Moderate edema (raised approximately 1 mm)
4	Severe erythema (beet redness) to eschar	Severe edema (raised more than 1 mm, and

formation preventing grading of erythema extending beyond exposure area)

6. Evaluation

All erythema grades and edema grades (24, 48 and 72 hours) separately for each test and control for each individual animal were calculated. The mean score of test article or control on each individual animal was calculated by dividing each of the totals by 15 (3 scoring time points × 5 sites). The overall mean for each test and control were calculated by adding the scores for the 3 animals and divide by 3. The difference between the overall mean score of the test article extracts and corresponding control extracts was calculated by subtracting the overall mean score for the control from the overall mean score for the test article extract. If the overall mean score of the test article extracts was less than the overall mean score of the corresponding control extracts, 0.0 was reported.

7. Results

All animals appeared normal throughout the study. Results of scores for individual animals appear in ATTACHMENT: Observations. All injection sites appeared normal immediately following injection. The overall mean difference for the extracts is summarized below:

Table 3: Results

Extract	Overall Test Group Mean	Overall Control Group Mean	Overall Mean Difference
SC	0	0	0
CSO	0	0	0

8. Conclusion

Under the conditions of this study, the test article (polar extract group) met the requirements of the test since the difference between test extract overall mean score and corresponding control overall mean score was 0; the test article (non-polar extract group) met the requirements of the test since the difference between test extract overall mean score and corresponding control overall mean score was 0.

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, T60122018-003.

STATEMENT OF QUALITY ASSURANCE ACTIVITIES

Phase Inspected	Date Inspected	Date Reported to Study Director	Date Reported to Management
Injection	04/12/2018	04/12/2018	04/12/2018
Study Data Review	04/15/2018	04/15/2018	04/15/2018
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: OBSERVATIONS

Animal Number	Sex	Body Weight (kg)	Group	Scoring Interval											
				24 Hours				48 Hours				72 Hours			
				Test		Control		Test		Control		Test		Control	
				ER	ED	ER	ED	ER	ED	ER	ED	ER	ED	ER	ED
A091	Male	3.14	SC	0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
			CSO	0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
A093	Male	3.13	SC	0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
			CSO	0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
A083	Male	2.80	SC	0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
			CSO	0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0

ATTACHMENT: ILLUSTRATION OF TEST ARTICLE