EASTDERM HYDROGEL WOUND DRESSING

<Lot No.: 2016.01.18.001>

White Rabbit Intracutaneous Irritation Test

FINAL REPORT

Sponsor: **Easting Biotech Co Ltd**
Testing Institution: **SGS Taiwan Ltd.**
Ultra Trace &Industrial Safety Hygiene
Report No.: **UB/2016/30966A-03**

Note:
1. The content of this report is invalid if it is not presented as the entire report.
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STUDY SCHEDULE

White Rabbit Intracutaneous Irritation Test
EASTDERM HYDROGEL WOUND DRESSING

Report No.:	UB/2016/30966A-03
Test Article Received Date:	2016.03.16
Experimental starting date:	2016.04.22
Experimental completion date:	2016.04.28
Study completion date:	See Study Director's signature date in the report

ADDRESS INFORMATION

Testing Facility/Test Site

Name: SGS TAIWAN LTD. Ultra Trace & Industrial Safety Hygiene
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Name: Biocompatibility Lab. of LEON Biotech. Co., Ltd.
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Study Director

Name: Benson Liu
Address: No. 38, Wu Chyuan 7th Rd., New Taipei Industrial Park, Wu Ku Dist., New Taipei City, 14890, Taiwan.

Sponsor

Name: Easting Biotech Co Ltd
Address: 3F., No.49, Xiwei St., Sanchong Dist., New Taipei City 24155, Taiwan (R.O.C.)

INFORMATION FOR TEST ARTICLE

台灣檢驗科技股份有限公司
SGS Taiwan Ltd.

INFORMATION FOR ☒TEST ARTICLE/ ☐CONTROL ARTICLE

Sponsor Company Name	Easting Biotech Co Ltd
Sponsor Address	3F., No.49, Xiwei St., Sanchong Dist., New Taipei City 24155, Taiwan (R.O.C.)
Name of Test Article/ Control Article	EASTDERM HYDROGEL WOUND DRESSING
Amount (Note 2)	A、Quantity/Unit: 15/pcs (e.g.10ml / bottle * 6 bottles) B、☒One Test (No Retention)☐Two Test (For Retention) C、Packing Condition: ☐In Bulk ☒Intact Packing
Sterilization	Has been Sterilized ☐NO ☒YES, If Yes, Please Select the Following Method, ☐EO Sterilization☒Gamma Sterilization☐Steam Sterilization☐Other_____
Expiry Date (Note 3)	☒Expiry Date: 2019 . 01 . 18 (YYYY.MM.DD) ☐Not Provided.
Batch/Lot Number	☒Specific Number：2016.01.18.001 ☐Not Provided.
Sample Description	A、Major Components: Hydrogel, Polyurethane Film B、Purity: N.A. C、Concentration: N.A. D、Stability：N.A E、Color : Translucent F、External Features: ☐Liquid ☐Irregular ☐Powder ☐Granule ☒Flat ☐Other:_____ G、Solvent and Solubility ：N.A
Attachment(Note 4)	☐Certificate of Analysis ☐Material Safety Data Sheet ☐Stability Test Result ☐Others：_____ ☒No Attachment (Note4)
Storage Condition	☒Room Temperature☐2~8℃☐-10~-25℃☐Others_____
Others	N.A

Test Article No.
UB/2016/30966
It will be labeled by SGS sample receiving personnel.

Note1. Above all information including the blanks which sponsor cannot provide are disclosure by the sponsor.
Note2. If the sponsor doesn't provide the retention of test article/control article, the retention of a reserved test article/control article from each batch of test article /control article is the responsibility of the Sponsor.
Note3. Should prepare extra amount of the same lot test/control article for the retention. For retention, if the effective period is less than 5 years, the test article/control article will be retained till the expiry date. If the expiry date is longer than 5 years, the test article/control article will be retained for 5 years only. If the expiry date remained incomplete, it mentions that sponsor will agree that test facility will determine the earliest date, e.g. Exp. Date: 2015(YYYY), sponsor didn't identify the MM/DD, the expiration date should be 2015.01.01.
Note4. Sponsor should determinate, document and confirm the identity, strength, purity, stability, composition, method of synthesis, fabrication, derivation or other characteristics of the test article/control article before study. If the sponsor cannot provide the information, determination and documentation of the test article/control article are the responsibility of the Sponsor.
Note5. Note 'N/A' or 'N.A'if not applicable. Do not leave blank.
Note6. Test article and control article should be filled individually in "INFORMATION FOR TEST ARTICLE/CONTROL ARTICLE".
Note7. GLP test：(1) Corrections and additions to a final report should be in the form of amendments. Amendments should clearly specify the reason for the corrections or additions. Lot number, test article photos and raw data cannot be amended by sponsor's requirement. (2)One protocol only can generate one report except for translation version. If the report with more than two languages version, we only issue English version protocol. We only issue amendment or additional language GLP report within three years and non-GLP report within one year. (3)The GLP compliance statement will state that we follow TFDA GLP and TAF OECD GLP norm unless sponsor's requirement. SGS Taiwan Ltd. UTIS have acquired Good Laboratory Practice Statement of Compliance from TFDA and TAF .
Note8. Please write down it carefully and in detail. "INFORMATION FOR TEST ARTICLE/CONTROL ARTICLE" will be placed in the protocol and report along with a copy of this official document. If the information is not clear, we will exclude them from GLP statement.

Sponsor Signature/ Date： 王以仁 2016/03/16

版次：3.5 試驗物質/對照物質資料表 Information For Test Article/Control Article
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SIGNATURE OF STUDY PERSONNEL

White Rabbit Intracutaneous Irritation Test
EASTDERM HYDROGEL WOUND DRESSING

Study Director: Benson Liu 2016.05.11

Benson Liu / SGS Taiwan Ltd. Date

Facility Manager: 2016.05.11

Yuanmin Wen / SGS Taiwan Ltd. Date

OBJECTIVE

The constituent materials of medical devices are considered potentially produce irritation. When direct contact with human tissues is anticipated, medical device should be carefully tested for biocompatibility according to the nature and duration of the contact to avoid potential physiological damage caused by irritative substances produced or contaminated during manufacture. Test procedure of *in vivo* is described in ISO 10993-10; albino rabbit use is suggested to evaluate the possibility of irritant reaction.

The test was performed following ISO 10993-10 and Leon Biotech. Co., Ltd. internal document of standard operating procedure SOP-T03, to investigate the response of intracutaneous irritation of "EASTDER HYDROGEL WOUND DRESSING" extract on New Zealand White Rabbits.

EXPERIMENTAL DESIGN

1. Test System

A. Species/ Strain: New Zealand White Rabbit (NZW)
B. Resource: Taiwan Livestock Research Institute (based on Leon Biotech SOP-Q02)
C. Reason: According to ISO 10993-10
D. Body weights /Age: >2 kg/ 2-12 month
E. Sex: Female. The female animals were nulliparous and not pregnant.
F. Number: 3
G. Quarantine/ acclimation: Once animals were introduced in-house, they were subjected to quarantine and acclimatize before treatment. Animals were selected based on health status by qualified staff. (according to Leon Biotech SOP-A02)
H. Animal restraint: The restraint of animals was according to Leon Biotech internal document of standard operating procedure SOP-T00
I. Identification
(1)Individual identification: Animals were identified by ear-marking.
(2)Group identification: Cages were properly labeled for identification including species/ strain, sex, in-housing date, IACUC number, animal I.D. number.
J. Housing condition
(1)Environment temperature: 20~26℃
(2)Humidity: 30~70%
(3)Cage and animal number: 1 animals/cage
(4)Fodder/ Supply: Lab Diet; *ad libitum*
(5)Drinking water/ Supply: Tap water; *ad libitum*

2. Reagents

A. 0.9% normal saline (Tai Yu Pharmaceutical Co., Ltd. Lot No.QB0604)
B. Cottonseed oil (SIGMA C7767, Lot No.MKBS9702V)

3. Extraction

According to ISO 10993-12 guidelines and Leon Biotech. Co., Ltd. internal document of standard operating procedure SOP-T01, the ratio of the test article to the extractant was 6cm^2/mL. In this study, 204 cm^2 test article was immersed in 34mL of 0.9% saline (Tai Yu Pharmaceutical Co., Ltd.) and 204 cm^2 test article was immersed in 34mL cottonseed oil (SIGMA C7767). Extract condition of 72±2 hours at 37℃ with constant agitation were executed in this study. The pH adjustment, filtration and centrifugation were not conducted. After polar and non-polar extraction, the appearance of extracts were not different from the control solution, clear. The extracts were not stored longer than 24 hours.

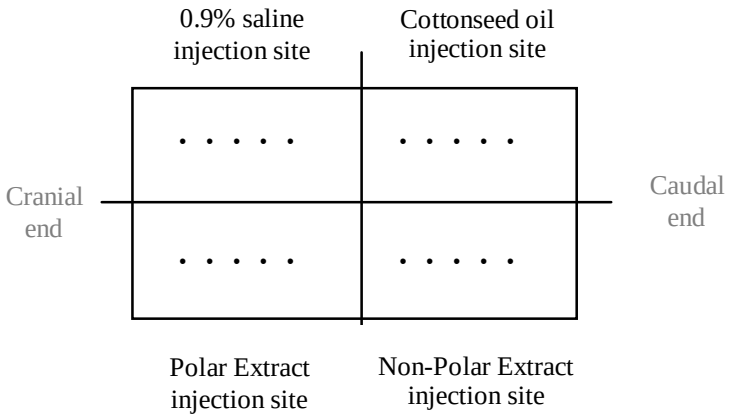
4. Grouping

Test group	Control group
3 animals	
Polar extract of test article	0.9% Saline
Non-polar extract of test article	Cottonseed oil

Note: The control solution and the test article extract were applied on different regions of the same rabbit.

5. Test Method

A. Within a 4 h to 18 h period before testing, furs of NZW rabbit backside from scapula to middle back were clipped with an electric animal shaver. Clipped zone are about 8cm x 15cm to exposure skin surface along the spine to execute intra-dermal injection. Animals with scratches or skin diseases in the clipped zone were rejected from study.



B. On the treatment day, a maker pen was used to divide clipped zone into four regions, and there were five injection site of each test and control group (see figure above).

C. Test article and control solution administration
   (1) Control solution was conducted in the right side of clipped zone with 0.9% saline in the anterior and cottonseed oil in the posterior region.
   (2) Test article extract was conducted in the left side of clipped zone with polar extract in the anterior and non-polar extract in the posterior region.
   (3) Each injection site was injected 0.2 mL solution.

D. Irritant reaction evaluation
   (1) The dermal reactions at the treated areas were observed and recorded based on "Grading system for intracutaneous reaction" (see Table 1) at the time points of 24^{th}, 48^{th} and 72^{nd} hours after administration.
   (2) The observation items included erythema, oedema, irritation, corrosion, recovery and other toxicity reactions.

E. Determination of dermal reaction
   (1) In the end of the test, each region in one rabbit, grades of erythema and oedema were summed and then divided by 15 (3 grading time points multiply 5 injection sites). To determine the mean score for each test sample and each corresponding blank, add the scores for the three animals and divide by three. The requirements of the test will be met if the differs between test article and control solution mean scores less than 1.0.
   (2) If at any observation period the average reaction to the test article is questionably greater than the average reaction to control side, the test will repeat using three additional rabbits.

RESULTS

During the study, there were no significant clinical signs and gross findings in either the control or test group, and there were no mortalities also.

1. Five injection site grades in clinical observation of individual rabbit were as below (polar group).

Treated article	Sex	Animal No.	Items for Grading	Clinical Observation (time point/h)		
				24	48	72
Test article Polar extract	F	RB-160218-07	Erythema and eschar formation	0	0	0
			Oedema formation	0	0	0
	F	RB-160218-11	Erythema and eschar formation	0	0	0
			Oedema formation	0	0	0
	F	RB-160218-12	Erythema and eschar formation	0	0	0
			Oedema formation	0	0	0
0.9% normal saline (control solution)	F	RB-160218-07	Erythema and eschar formation	0	0	0
			Oedema formation	0	0	0
	F	RB-160218-11	Erythema and eschar formation	0	0	0
			Oedema formation	0	0	0
	F	RB-160218-12	Erythema and eschar formation	0	0	0
			Oedema formation	0	0	0

2. Five injection site grades in clinical observation of individual rabbit were as below (Non-polar group).

Treated article	Sex	Animal No.	Items for Grading	Clinical Observation (time point/h)		
				24	48	72
Test article Non-polar extract	F	RB-160218-07	Erythema and eschar formation	5	4	4
			Oedema formation	2	3	2
	F	RB-160218-11	Erythema and eschar formation	5	2	2
			Oedema formation	5	2	1
	F	RB-160218-12	Erythema and eschar formation	10	5	4
			Oedema formation	10	8	4
Cottonseed oil (control solution)	F	RB-160218-07	Erythema and eschar formation	5	3	3
			Oedema formation	2	0	0
	F	RB-160218-11	Erythema and eschar formation	2	1	1
			Oedema formation	0	0	0
	F	RB-160218-12	Erythema and eschar formation	10	5	5
			Oedema formation	10	5	5

F: Female

Some erythema and oedema were recorded in non-polar group, but there were no difference between test and control group. It could be the background reaction of cottonseed oil and animal individual difference.

$$Final\ scores = \frac{\text{Total scores of test group}}{15 * 3} - \frac{\text{Total scores of control group}}{15 * 3}$$

Final calculated score value was less than 1.0 in both test groups (polar group: 0/45-0/45=0; non-polar group: 78/45-57/45=0.466), which indicated negative results.

CONCLUSION

The study results showed that a single application of “EASTDER HYDROGEL WOUND DRESSING” extract induced neither significant clinical signs nor dermal gross changes on New Zealand White Rabbits at each time point. Furthermore, the final score value of test article extract was less than 1.0. Therefore, single intracutaneous application with 0.2 mL of “EASTDER HYDROGEL WOUND DRESSING” extract did not cause irritation on New Zealand White Rabbits.

REFERENCES

1. Acute dermal irritation/corrosion, OECD guideline for the testing of chemicals. #404 (2015) OECD.
2. Biological evaluation of medical devices- Part 2: Animal welfare requirements. ISO 10993-2:2006.
3. Biological evaluation of medical devices-Part 10: Tests for irritation and skin sensitization, ISO 10993-10:2010.
4. Biological evaluation of medical devices- Part 12: Sample preparation and reference materials. ISO 10993-12:2012.

TABLES

1. Grading System for Intracutaneous Reaction

Reaction	Primary Irritation Score
Erythema and eschar formation	
· No erythema	0
· Very slight erythema (barely perceptible)	1
· Well-defined erythema	2
· Moderate erythema	3
· Severe erythema (beet redness) to eschar formation preventing grading or erythema	4
Oedema formation	
· No oedema	0
· Very slight oedema (barely perceptible)	1
· Well-defined oedema (edges of area well-defined by definite raising)	2
· Moderate oedema (raised approximately 1 mm)	3
· Severe oedema (raised more than 1 mm and extending beyond exposure area)	4

TEST ARTICLE PHOTO

UB/2016/30966