Ataraxis Disposable Face Towelette

White Rabbit Skin Irritation Test

Test Report

Client	**Ataraxis Medical Supply Co. Ltd.**
Testing Institution	**Biocompatibility Lab. of LEON Biotech. Co., Ltd.**
Report No.	**R-SR-KL20230609**

Note:
1. The content of this test report is invalid if it is not presented as the entire test report.
2. Any unauthorized alteration, forgery or falsification of the content or appearance of this test report is unlawful and offenders may be prosecuted to the fullest extent of the law.
3. The results shown in this test report refer to the test article(s) tested only.

Contents

Schedule

Study	White rabbit skin irritation test
Test article	Ataraxis Disposable Face Towelette
Service No.	KL20230609
Experimental starting date	2023.07.04
Experimental completion date	2023.07.07
Report Date	See Approved Signatory's signature date in the report

Study Personnel

Participants	W. L. Yeh, Y. C. Yang, J. M. Chen

Test Institution

Name	Biocompatibility Lab. of LEON Biotech. Co., Ltd.
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Contact	Ming-Yang Tsao (07) 841-9003 service@leon-bio.com.tw

Client

Name	Ataraxis Medical Supply Co. Ltd.
Address	2F., No. 14, Ln. 122, Sec. 2, Xiwan Rd., Xizhi Dist., New Taipei City, Taiwan

Test Article Information

Name	Ataraxis Disposable Face Towelette
Supplier/Manufacturer	Ataraxis Medical Supply Co., Ltd.
Model Number (REF)	N/A
Lot No.	45275381
Permit No.	N/A
Manufacture Date	2023.05.31
Expiry Date	2026.05.31
Storage Condition	Room temperature
Sterilization Condition	Non
Package	Plastic bag
Main Ingredient	Rayon
Purity	N/A
Concentration	N/A
Stability	N/A
Homogeneity	N/A
Surface Area	400 cm^2/piece (human contact part)
Thickness	Non provided
Weight	Non provided
Appearance Description	Sheet; White color
Category	Medical device
Location of Origin	Taiwan

Pre-treatment	N/A
Attachment	Non
Other Notification	N/A
✦ **Sponsor, who provided test facility with the test article information, will take full responsibility for all the facts of it.**	
Received Date	**2023.06.07**
Test Article No.	**KL20230609-a**

Archiving

All the study-related records, raw data, and the test report were kept in archives room of LEON Biotech. for 6 years.

Archiving List	
Records	Application form (SOP-Q07-F01) Work order (SOP-Q07-F02) Test article information (SOP-Q10-F03) Test article control form (SOP-Q10-F02) and other supplementary records
Raw Data	Test article extraction record (SOP-T01-F01) White rabbit skin irritation test data sheet (SOP-T04-F01)
Test Report	Test Report Test Report amendment (if necessary)

Approved Signatory

M. Y. T

2023.7.24

Ming-Yang Tsao / LEON Biotech. Co., Ltd. **Date Completed**

Objective

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 hypersensitive substances produced or contaminated during manufacture. The study was performed in accordance with ISO 10993-23 and internal document of standard operating procedure SOP-T04, to investigate the response of skin irritation of “Ataraxis Disposable Face Towelette” on New Zealand White Rabbits.

Test System

Species / Strain	New Zealand White Rabbit (NZW)
Resource	Taiwan Livestock Research Institute (TLRI) (Animal purchasing procedure was based on SOP-Q02)
Reason	According to ISO10993-23
Body weight	>2 kg
Sex	Female The female rabbits were nulliparous and non-pregnant.
Numbers	3
Quarantine / Acclimation	Once animals are introduced in-house, they are subjected to quarantine and acclimatize before treatment. Animals are selected based on health status by qualified staff. (according to SOP-A02)
Animal restraint	The restraint of animals was according to internal document of standard operating procedure SOP-T00.
Identification	
Individual identification	Animals are identified by ear-marking.
Cage identification	Cages are properly labeled for identification including species/strain, sex, in-housing date, IACUC number, animal I.D. number.
Housing condition (according to SOP-A01)	
Environment temperature	23±3℃
Humidity	30~70%
Cage and animal number	1 animal/cage
Fodder / Supply	Lab Diet #5326; *ad libitum*
Drinking water / Supply	Tap water from Taiwan water corporation purified by water purifier; *ad libitum*

Material and Method

Reagent

1. 0.9% normal saline (Tai Yu Chemical and Pharmaceutical Co., Ltd. Lot No. XG2902)
2. Distilled water (Tai Yu Chemical and Pharmaceutical Co., Ltd. Lot No. XL0704)

Preparation

According to ISO 10993-12 guidelines and internal document of standard operating procedure SOP-T04 and SOP-T01, test article was applied to each test skin site directly.

Grouping

Test group	Control group
3 animals	
Test article	Absorbent gauze

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

Test Method

1. Preparation

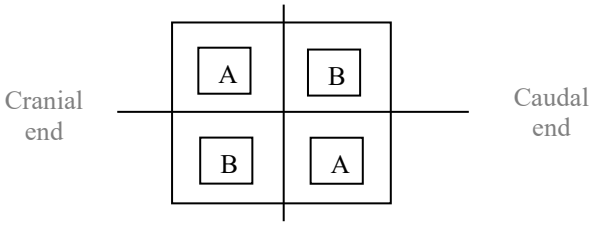
1.1. Within a 4 h to 24 h period before testing, furs of NZW rabbit backside from scapula to middle back were clipped before test. Clipped zone was about 10 cm × 15 cm to exposure skin surface.

1.2. A marker pen was used to divide clipped zone into four regions (see figure below). Animals with scratches or skin diseases in the clipped zone should be rejected from study.

2. Administration of test article and control article

2.1. Test article (cut to 2.5 cm × 2.5 cm) were moistened sufficiently with 0.9% normal saline (Lot No. XG2902) and applied on both B sites (see figure below). In addition, A sites were

applied with sterile gauze (2.5 cm × 2.5 cm) (Lot No. 20270118) saturated with 0.9% normal saline (Lot No. XG2902) for control. The application sites were wrapped with elastic and porous bandages.



2.2. After 4 hours, the tapes and gauzes were all removed, and then the test article and control item were washed off with distilled water.

3. Irritant reaction evaluation

3.1. The dermal reactions at the treated areas were observed and recorded at 1±0.1h, 24±2h, 48±2h and 72±2h after the removal of the gauzes of test and control group. The observation items included erythema, oedema, and other toxicity reactions (Table 1).

4. Determination of dermal reaction

4.1. After a single dose treatment, the skin responses at 24±2h, 48±2h and 72±2h after the gauze removed were checked and evaluated, according to “Score System of Skin Reaction” described in Table 1.

4.2. Primary Irritation Index (PII) was calculated based on the erythema and oedema scores for evaluating dermal response (Table 2).

5. Reliability check

5.1. Positive control shall be performed at least once every six months.

5.2. Sodium dodecyl sulfate (SDS) was used as the positive control substances.

5.3. The result of the most recent positive control test was embedded in the test report.

Result

1. Grades in clinical observation of individual rabbit were as below

Animal ID / Body Weight (kg)	Sex	Items for Grading	Test (Site B)								Control (Site A)							
			Clinical Observation Time Point (h)								Clinical Observation Time Point (h)							
			1±0.1		24±2		48±2		72±2		1±0.1		24±2		48±2		72±2	
			1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
RB-230309-08	F	Erythema and eschar formation	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3.5352		Oedema formation	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RB-230209-07	F	Erythema and eschar formation	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3.1374		Oedema formation	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RB-221013-10	F	Erythema and eschar formation	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3.8726		Oedema formation	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Test and Control were performed in duplicate on each animal.
F: Female

Primary irritation index (PII) was calculated as shown below.

$$PIS_1 = \frac{0-0}{6} = 0 \qquad PIS_2 = \frac{0-0}{6} = 0 \qquad PIS_3 = \frac{0-0}{6} = 0$$

$$\text{Primary irritation index (PII)} = \frac{0+0+0}{3} = 0$$

◆ PIS: primary irritation score for individual animals

The results showed that there was no obvious erythema and oedema finding in either the test or control group. There were neither mortalities nor other adverse effects observed on all test animals during the test period. Furthermore, the primary irritation index (PII) value is 0, which indicates the

negative results.

Reliability Check

The positive control study was finished on Apr. 28th, 2023. According to ISO 10993-23 guidelines, positive control shall be performed at least once every six months. Sodium dodecyl sulfate (Sigma L5750, Lot. No. SLCG5986) were used for positive control substances. The method for the positive control assay is identical to the method described above in this study. For the study 20% sodium dodecyl sulfate was used.

The scores were 3 of erythema and oedema at 24±2 hours, scores were 2 of erythema and oedema at 48±2 hours, scores were 2 of erythema and 1 to 2 of oedema at 72±2 hours. Animals in the positive control group exhibited well-defined to moderate erythema and slight to moderate oedema at the test site, therefore the primary irritation index (PII) value was 4.5, which indicates the positive results.

Conclusion

The results showed that test article treated site did not show any significant erythema and edema, which was compared to the concurrent control site. There was also no mortality in the study. Furthermore, the primary irritation index (PII) value is 0. It represented that the test article was non-irritant under this test condition. Therefore, a single topical application of "Ataraxis Disposable Face Towelette" did not cause skin irritation.

Table

1. Score System of Skin Reaction

Reaction	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 of erythema	4
Other adverse changes at the skin sites shall be recorded and reported.	
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
Maximal possible score for irritation	8
Other adverse changes at the skin sites shall be recorded and reported.	

2. Evaluation Table of Single Dermal Irritation

Mean score	Response category
0 to 0,4	negligible
0,5 to 1,9	slight
2 to 4,9	moderate
5 to 8	severe

PIS = $S_T - S_C / 6$ (Two test sites, three observation time points)

PIS = Primary Irritation Scores of each animal

S_T = Total scores of two test sites in three observation time points

S_C = Total scores of two control sites in three observation time points

PII = (Total PIS of 3 animals)/3

Reference

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 10993-2:2022.
3. Biological evaluation of medical devices- Part 12: Sample preparation and reference materials. ISO 10993-12:2021.
4. Biological evaluation of medical devices- Part 23: Tests for irritation. ISO 10993-23:2021.

Test Article Photo