FINAL REPORT

Skin Irritation Test in New Zealand White Rabbits of Taglus Standard Thermoforming Foils as per ISO 10993-23:2021(E).

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Study No.: 073/434

STUDY SPONSOR AND APPLICANT:
Vedia Solutions
Division of Laxmi Dental Export Pvt Ltd
103, Akruti Arcade, JP Road
Opp A H Wadia School, Andheri West
Mumbai 400053

REPORT ISSUED DATE: 07 January 2022

FINAL REPORT

PRODUCT NAME:

Taglus Standard Thermoforming Foils

STUDY TITLE

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STUDY DIRECTOR AUTHENTICATION STATEMENT

Study No. : **073/434**

Study Title : **Skin Irritation Test in New Zealand White Rabbits of Taglus Standard Thermoforming Foils as per ISO 10993-23:2021(E)**

This study was performed in accordance with the mutually agreed study plan and GLR Laboratories Private Limited's standard operating procedures, unless otherwise stated, and the study objective was achieved. I accept overall responsibility for the technical conduct of the study, as well as for the interpretation, analysis, documentation and reporting of results. This report provides a true and accurate record of the results obtained.

This study was performed in compliance with OECD Principles of Good Laboratory Practice* ENV/MC/CHEM (98)17 (Revised 1997, issued January 1998) and applicable regulatory requirements including the US Food and Drug Administration's GLP regulations, 21 CFR 58 (subparts B to G and J).

07 Jan 2022

Mr. K. Sakthivel, MSc (Biotech)
Study Director
GLR Laboratories Private Limited

Study Completion Date

*The identity and composition of the test item are the responsibilities of the Sponsor.

QUALITY ASSURANCE STATEMENT

Study No. : **073/434**

Study Title : **Skin Irritation Test in New Zealand White Rabbits of Taglus Standard Thermoforming Foils as per ISO 10993-23:2021(E)**

The Quality Assurance (QA) of GLR Laboratories Private Limited verified the Study Plan, including any amendments, inspected the critical study phases, audited the raw data and report of this Study as per in-house Standard Operating Procedures (SOPs) for compliance with the OECD Principles of Good Laboratory Practice (as revised in 1997) [ENV/MC/CHEM (98)17], and for compliance with relevant regulatory requirements.

During the Study, the following study-related inspections/audits were performed on the following dates and reported to the Study Director and Test Facility Management. Besides the below, process and facility inspections were also carried out periodically at this Test Facility by auditor(s) of the QA, as per in-house SOPs, which may have relevance to this study.

S. No.	Type(s) of Study Inspection/Audit	Date(s) of Inspection/Audit	Phase(s) of Study inspected/audited	Date(s) of Reporting to Management and Study Director (Inspection No.)
1	Study Plan Verification	19 November 2021	Draft Study Plan	19 November 2021 (SBI/073/434/001)
2	Study Plan Verification	26 November 2021	Definitive Study Plan	26 November 2021 (SBI/073/434/002)
3	In-life Phase Inspection	14 December 2021	Test Item Extracts Application	14 December 2021 (SBI/073/434/003)
4	In-life Phase Inspection	17 December 2021	Grading of Skin Reactions	17 December 2021 (SBI/073/434/004)
5	Report Audit	04 January 2022	Draft Report	04 January 2022 (SBI/073/434/005)
6	Report Audit	07 January 2022	Final Report	07 January 2022 (SBI/073/434/006)

The QA has determined that the methods, procedures, observations, and reported results are accurately and completely described and that the reported results are based on the Study Plan and the pertinent raw data generated during the course of the Study. The Study Director's GLP compliance statement is supported.

07 JAN 2022

Dr. G. Velmani, M Pharm, PhD
Executive Quality Assurance
GLR Laboratories Private Limited

Date

TEST FACILITY MANAGEMENT STATEMENT

Study No. : **073/434**

Study Title : **Skin Irritation Test in New Zealand White Rabbits of Taglus Standard Thermoforming Foils as per ISO 10993-23:2021(E)**

This is to certify that, the Test Facility Management appointed the Study Director and provided all necessary facilities and resources for the proper conduct of this study, in compliance with the Principles of OECD Good Laboratory Practice (GLP), as per the recommendations of the OECD (Council Act [C (97) 186 (Final)]) and as adopted in the procedures promulgated by the National GLP Compliance Monitoring Authority, Government of India.

07 Jan 2022

Dr. S. S. Murugan, PhD
Test Facility Management
Managing Director
GLR Laboratories Private Limited

Date

SUMMARY

Skin irritation potential of Taglus Standard Thermoforming Foils, supplied by Vedia Solutions, was evaluated in male New Zealand White rabbits.

The test item, Taglus Standard Thermoforming Foils is a surface device which comes in contact with mucosal membrane. The dimension of the test item are: - diameter: 12.5 cm and thickness: 0.8 mm. The duration of contact is less than 24 hours (limited).

The test item was extracted at a ratio of 3 cm^2/mL (since the thickness of the test item is more than 0.5 mm) in polar solvent (physiological saline) as well as non-polar solvent (Cottonseed oil) respectively at 37 ± 1 °C for 72 h and 03 min under sterile conditions. The total surface area of the test item is 245 cm^2 (as calculated in our laboratory). Polar extract was prepared by extracting 245 cm^2 of test item in 81.7 mL of physiological saline. Similarly, non-polar extract was prepared by extracting 245 cm^2 of test item in 81.7 mL of Cottonseed oil. Solvent controls were also subjected to same extraction conditions. This fulfills the requirements of ISO 10993-12:2012(E) and ISO 10993-12:2021(E).

At the end of extraction, extract and solvent controls were clear without any colour change or particulates. No additional processing such as filtration, centrifugation, pH adjustments or any other processing were made. No changes were observed in retrieved test item. The extracts and solvent controls were transferred to sterile containers and stored at room temperature. All extracts and solvent controls were used within 2 h and 27 min of preparation and were considered stable during this time.

About 18 h and 30 min prior to application, fur on all the rabbits were closely clipped off their backs, allowing sufficient distance on both sides of the spine for application of test item extracts and solvent controls. The test item extracts (0.5 mL) was loaded on the absorbent gauze measuring 6.25 cm^2 (2.5 cm x 2.5 cm) and placed topically on the fur clipped test sites of six male rabbits (3 animals each for polar and non-polar extracts). Similarly, 0.5 mL of solvent controls was loaded on the absorbent gauze measuring 6.25 cm^2 (2.5 cm x 2.5 cm) and placed topically on the fur clipped control sites. The patches were loosely held in contact with the skin by semi-occlusive dressing with means of non-irritant adhesive tape for 4 h. After 4 h the patch was removed. No test item extract residues were observed. The test sites were marked with a non-irritant permanent ink.

Animals were observed for three consecutive days for morbidity, mortality, skin reactions and abnormal clinical signs and symptoms following the patch removal. The skin reactions were visually scored according to ISO 10993: Part 23:2021(E) at 1, 24, 48 and 72 h.

Positive control trials for irritation are carried out periodically at GLR Laboratories Private Limited in New Zealand White rabbits using sodium laury1 sulphate in compliance with regulatory guidelines. The trial completed on 20 October 2021 gave a "moderate irritant" reaction. No response was observed in solvent control treated animals. Therefore, the assay was considered valid.

No mortality or morbidity was observed in the experimental animals. A gradual increase in body weight was observed in all the animals at the end of the experiment. No signs of clinical toxicity or overt toxicity was observed in any of the animals. Hence, gross pathology and histopathology was not performed. No local skin irritation was observed at the test site in any of the animals and the primary irritation index obtained was '0'.

Based upon the results obtained in this study and in line with ISO 10993-23:2021(E) the given test item, Taglus Standard Thermoforming Foils, supplied by Vedia Solutions, is considered a non-irritant in New Zealand white rabbits under the conditions of the present study.

INTRODUCTION

Biocompatibility testing is a regulatory requirement for demonstrating the preclinical safety of medical devices. This is evaluated in line with the standard guideline, ISO 10993-1:2018(E), Biological Evaluation of Medical Devices - Part 1, Evaluation and Testing within a Risk Management Process. This standard describes the necessity to select a suitable test method for biocompatibility evaluation.

Skin irritation is a key toxicity endpoint to assess biocompatibility of medical devices. An assessment is made of the potential of the material under test to produce dermal irritation in rabbits following topical application.

OBJECTIVE

To determine the skin irritation potential of the test item in New Zealand white rabbits.

STUDY DATES

Study Start Date	26 November 2021
Experiment Start Date	07 December 2021
Experiment Completion Date	17 December 2021

The study completion date is the date the final report is signed by the Study Director.

TEST AND CONTROL ITEM DETAILS

The test item, Taglus Standard Thermoforming Foils was received at GLR Laboratories Private Limited on 23 October 2021 and stored at room temperature (20.1 to 24.1 °C) until used.

The following test item information provided by the sponsor were considered adequate.

Test Item	Taglus Standard Thermoforming Foils
Batch / Lot No.	12029092-1
Manufacture Date	29 September 2021
Expiry Date	20 September 2024
Appearance	Transparent disk
Ingredients	PETG (Polyethelene Tertamethylene Glycol)
Temperature Stability	37 °C
Sterility	Non-Sterile

CONTROL ITEM DETAILS

Positive Control	20% Sodium Lauryl Sulphate (SLS)	
	Manufacturer	Sigma Aldrich
	Batch No.	0000009635
	Expiry date	August 2022
	Positive control trials for irritation are carried out periodically at GLR Laboratories Private Limited in New Zealand White rabbits using sodium lauryl sulphate in compliance with regulatory guidelines (OECD 404, ISO 10993-10:2010(E) and ISO 10993-23:2021(E)). The trial completed on 20 October 2021 gave a "moderate irritant" reaction (Appendix 1).	
Solvent Controls	Physiological saline	
	Manufacturer	Eurolife Healthcare Pvt. Ltd.
	Batch No.	10210671B
	Expiry Date	September 2024
	Appearance	Colourless clear solution
	Cottonseed oil	
	Manufacturer	Sigma-Aldrich
	Lot No.	MKCM9272
	Expiry Date	October 2026
	Appearance	Yellow coloured viscous liquid

The test item was handled with all necessary protective clothing and all recommended safety and sterile measures were followed. The identity, composition stability and characteristics of the test item is the responsibility of the sponsor. No analysis was performed at GLR Laboratories Private Limited, to confirm it.

Description of the test item

The test item, Taglus Standard Thermoforming Foils is a surface device which comes in contact with mucosal membrane. The dimension of the test item are: - diameter: 12.5 cm and thickness: 0.8 mm. The duration of contact is less than 24 hours (limited).

TEST SYSTEM

Species	*Oryctolagus cuniculus* (Rabbit)
Strain	New Zealand White
Weight range (g) (at the time of dosing)	2124.8 to 2360.0

Sex	Male
Source	Animals were procured from the supplier approved by, the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and were quarantined for 7 days. Supplier - VAB Biosciences #1-6-197/45/D, Bapuji Nagar Musheerabad, Hyderabad-500020
Number of animals used	6
Acclimatization period	7 days
Justification for animal use	Rabbits were selected because there is a large volume of background data on this species. Recommended in ISO 10993-23:2021(E) standard as an appropriate species to evaluate skin irritation of medical devices and by various regulatory authorities.

The test system was approved by the Institutional Animal Ethics Committee (IAEC) of GLR Laboratories Private Limited.

ANIMAL HUSBANDRY

Test room no.	12
Test room temperature (°C)	18.0 to 21.7
Relative humidity (%)	37 to 58
Housing	Animals were housed individually in standard rabbit cages.
Method of identification	Animals were identified using cage cards indicating cage no., study no., species, strain, animal no., sex, body weight, dose and individual ear marking.
Feed	Commercial rabbit pellet feed Supplier - VRK Nutritional Solutions D-47 & W-38, MIDC area, Miraj, Dist. Sangli- 416410, Maharashtra (India)
Water	Purified drinking water was provided *ad libitum*
Bedding material	No bedding material were used as rabbits are housed in stainless steel cages with mesh floors. A tray with sterilized paddy husk was used to collect the excreta and urine and were changed daily. This did not have direct contact with rabbits.

	Supplier - M/S K. Dhandapani 4/331, Old Mahabalipuram Road Kottivakkam, Chennai-600041 Tamilnadu (India)
Photoperiod	12 h: 12 h light and dark cycle
Contaminants	Contaminants, reasonably expected in feed or water supplies are not believed to influence the outcome of the study. Analysis of feed, water and bedding materials are carried out once in every 6 months and the results of the most recent analysis were placed in the study file.
Personnel	Appropriately qualified and trained personnel were involved in this study.
Selection of animals	Previously unused and healthy young adults were selected for this study.

TEST METHOD

Preparation of the test item and control item

The test item was extracted at a ratio of 3 cm^2/mL (since the thickness of the test item is more than 0.5 mm) in polar solvent (physiological saline) as well as non-polar solvent (Cottonseed oil) respectively at 37 ± 1 °C for 72 h and 03 min under sterile conditions. Solvent controls were also subjected to same extraction conditions. This fulfills the requirements of ISO 10993-12:2012(E) and ISO 10993-12:2021(E).

Extract	Extraction vehicle	Total Surface area of the test item (cm^2)	Volume of vehicle (mL)	Extract preparation start time	Extract preparation end time	Appearance of extracts
Extract 1	Physiological saline	245	81.7	09:05 a.m. on 11 Dec 2021	09:08 a.m. on 14 Dec 2021	Colourless clear solution, no particulates
Extract 2	Physiological saline	NA	10			Colourless clear solution, no particulates
Extract 3	Cottonseed oil	245	81.7			Yellow viscous liquid, no particulates
Extract 4	Cottonseed oil	NA	10			Yellow viscous liquid, no particulates

NA-Not applicable; Extraction time: 72 h and 03 min.

No additional processing such as filtration, centrifugation, pH adjustments or any other processing were made. No changes were observed in retrieved test item. The extracts and solvent controls were transferred to sterile containers and stored at room temperature. All extracts and solvent controls were used within 2 h and 27 min of preparation and were considered stable during this time.

Test procedure

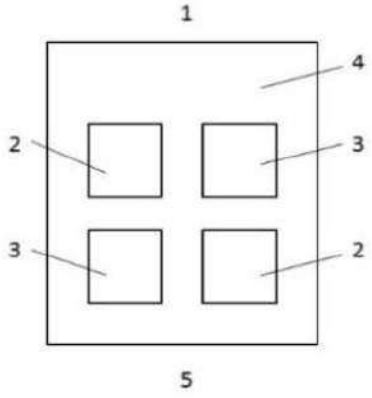
Justification for method of application	Specified in ISO 10993-23:2021(E) standard, skin irritation in rabbit is recommended as a suitable method to determine the biocompatibility of medical devices.

The animals with healthy intact skin were selected for this study. About 18 h and 30 min prior to application, fur on all the rabbits were closely clipped off their backs for an area of 10 cm x 15 cm on both sides of the spine.

Topical application

Test item extract (0.5 mL) was loaded on the absorbent gauze measuring 6.25 cm^2 (2.5 cm x 2.5 cm) which was placed as such on to the fur clipped area of rabbit skin, in the dorsal region on the left cranial end and right caudal end. Similarly, 0.5 mL of solvent controls was applied on the absorbent gauze (Make: The Ramaraju Surgical Cotton Mills Ltd; Batch No: 578/19; Expiry Date: July 2022) measuring 6.25 cm^2 (2.5 cm x 2.5 cm) which was placed as such on to the fur clipped area of rabbit skin, the right cranial end and left caudal end as shown in the following figure.

The patches were loosely held in contact with the skin by semi-occlusive dressing with means of non-irritant adhesive tape [Make: 3M India Limited; Batch No.: R05190315; Expiry Date: April 2024] for the duration of 4 h. No test item extracts residues were observed. The test sites were marked with non-irritant permanent ink.



1. Cranial end; 2. Test site; 3. Control site; 4. Clipped dorsal region; 5. Caudal end.
Source: ISO 10993-23:2021(E)

OBSERVATIONS

Mortality & Morbidity

Animals were observed for mortality and morbidity daily throughout the experiment.

Body Weight

Body weights of each animal were recorded prior to dosing and at the end of experiment.

Clinical observations

Animals were examined for signs of erythema and oedema. The responses were scored at 1 h, and then at 24 h, 48 h and 72 h following the patch removal.

Grading of skin reactions

Animals were macroscopically examined for signs of erythema and oedema, visually with naked eyes. Skin reactions were graded and recorded at 1 h, and then at 24 h, 48 h and 72 h following the patch removal according to ISO 10993-23:2021(E)

Skin reactions were recorded at each examination as shown in the table below.

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
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
Maximum possible score for irritation: 8	

Source: ISO 10993-23:2021(E)

In addition to the observation of irritation, all local toxic effects, such as defatting of the skin, and any systemic adverse effects (e.g., effects on clinical signs of toxicity and body weight), were recorded.

Euthanasia

Animals were euthanized by overdose of thiopental sodium injection at the end of the experiment.

Necropsy and Gross pathology

None of the animals were found dead or in moribund condition hence necropsy and gross pathology was not performed.

DATA EVALUATION

The skin irritation scores were evaluated in conjunction with the nature and severity of lesions, and their reversibility or lack of reversibility. The individual scores do not represent an absolute standard for the irritant properties of a material, as other effects of the test material are also evaluated.

After 72 h grading, all erythema grades plus oedema grades at 24 h, 48 h and 72 h were totalled separately for test item and control for each animal. The primary irritation score for an animal was calculated by dividing the sum of all the scores by 6 (two test/observation sites, three time points). The primary irritation index (PII) of the test item and control was obtained by adding the scores of the individual animals and dividing it by the total number of animals. The results were evaluated by calculating the difference between the primary irritation score of control and test item.

Based on the observations and primary irritation response, the test item was categorised as per the primary irritation index (Appendix 2).

ACCEPTANCE CRITERIA

The assay is considered valid as the following criterions are met:

1. Positive control trial conducted within the test facility indicated a clear positive result.

2. Solvent control used in the study gave a mean irritation score of 0.

RESULTS

Mortality & Morbidity

No mortality or morbidity occurred in any of the animals used in this study.

Body Weight

A gradual increase in body weight was observed in all the animals at the end of experiment. Individual body weight of the animals is given in Table 1.

Clinical observations

No signs of ill health or overt toxicity were observed in the experimental animals.

Grading of skin reactions

No local skin irritation was observed at the test site in any of the animals and the primary irritation index obtained was '0'. The individual score for erythema/eschar and oedema of the test site and control site after 1 h, 24 h, 48 h and 72 h following patch removal are given in Table 2 and 3 for all the animals. Mean irritation scores of grading and the difference in primary irritation index of test and control sites are given in Tables 4, 5 & 6.

CONCLUSION

Based upon the results obtained in this study and in line with ISO 10993-23:2021(E) the given test item, Taglus Standard Thermoforming Foils, supplied by Vedia Solutions, is considered a non-irritant in New Zealand white rabbits under the conditions of the present study.

REFERENCES

1. Biological Evaluation of Medical Devices - Part 1, Evaluation and Testing within a Risk Management Process, ISO 10993-1:2018(E).

2. Biological Evaluation of Medical Devices - Part 2, Animal Welfare Requirements, ISO 10993-2:2006(E).

3. Biological Evaluation of Medical Devices - Part 23, Tests for Irritation, ISO 10993-23:2021(E).

4. Biological Evaluation of Medical Devices - Part 12, Sample Preparation and Reference Materials, ISO 10993-12:2012(E).

5. Biological Evaluation of Medical Devices - Part 12, Sample Preparation and Reference Materials, ISO 10993-12:2021(E).

6. OECD Principles of Good Laboratory Practice. OECD Environmental Health and Safety Publications, Series on Principles of Good Laboratory Practice and Compliance Monitoring No. 1. ENV/MC/CHEM (98)17.

7. General Requirements for the Competence of Testing and Calibration Laboratories, ISO/IEC 17025:2017(E).

8. Use of International Standard ISO 10993-1, "Biological Evaluation of medical devices - Part 1: Evaluation and Testing within a risk management process". Guidance for Industry and Food and Drug Administration Staff. September 04, 2020.

Table 1: Individual body weights

Animal number	Sex	Individual body weights (grams)	
		At the time of dosing	At the end of experiment
1	male	2203.8	2227.0
2		2124.8	2151.2
3		2337.0	2362.2
4		2300.9	2323.6
5		2360.0	2385.2
6		2250.1	2273.0

Table 2: Individual grades of skin reactions

	Observation Time (h)	Individual score																	
		Animal number 1						Animal number 2						Animal number 3					
		T_1	T_2	T	C_1	C_2	C	T_1	T_2	T	C_1	C_2	C	T_1	T_2	T	C_1	C_2	C
Erythema and Eschar formation	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	48	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	72	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oedema formation	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	48	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	72	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

C_1-First control site; C_2-Second control site; C-Sum of C_1 & C_2
T_1- First test site; T_2- Second test site; T-Sum of T_1 & T_2
Source: ISO 10993-23:2021(E)

Table 3: Individual grades of skin reactions

	Observation Time (h)	Individual score																	
		Animal number 4						Animal number 5						Animal number 6					
		T_1	T_2	T	C_1	C_2	C	T_1	T_2	T	C_1	C_2	C	T_1	T_2	T	C_1	C_2	C
Erythema and Eschar formation	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	48	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	72	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oedema formation	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	48	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	72	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

C_1-First control site; C_2-Second control site; C-Sum of C_1 & C_2
T_1- First test site; T_2- Second test site; T-Sum of T_1 & T_2
Source: ISO 10993-23:2021(E)

Table 4: Calculation of primary irritation score at three time points

		Observation Time (h)	Individual score								
			Animal number 1			Animal number 2			Animal number 3		
			Score	Total Score	PI Score	Score	Total Score	PI Score	Score	Total Score	PI Score
Test (T)	Erythema and Eschar formation	24	0	0	0	0	0	0	0	0	0
		48	0			0			0		
		72	0			0			0		
	Oedema formation	24	0			0			0		
		48	0			0			0		
		72	0			0			0		
Control (C)	Erythema and Eschar formation	24	0	0	0	0	0	0	0	0	0
		48	0			0			0		
		72	0			0			0		
	Oedema formation	24	0			0			0		
		48	0			0			0		
		72	0			0			0		

Total score = Sum of all the scores at test site (or) solvent control site;
Primary Irritation (PI) Score = Total score divided by 6;
Source: ISO 10993-23:2021(E)

Table 5: Calculation of primary irritation score at three time points

		Observation Time (h)	Individual score								
			Animal number 4			Animal number 5			Animal number 6		
			Score	Total Score	PI Score	Score	Total Score	PI Score	Score	Total Score	PI Score
Test (T)	Erythema and Eschar formation	24	0	0	0	0	0	0	0	0	0
		48	0			0			0		
		72	0			0			0		
	Oedema formation	24	0			0			0		
		48	0			0			0		
		72	0			0			0		
Control (C)	Erythema and Eschar formation	24	0	0	0	0	0	0	0	0	0
		48	0			0			0		
		72	0			0			0		
	Oedema formation	24	0			0			0		
		48	0			0			0		
		72	0			0			0		

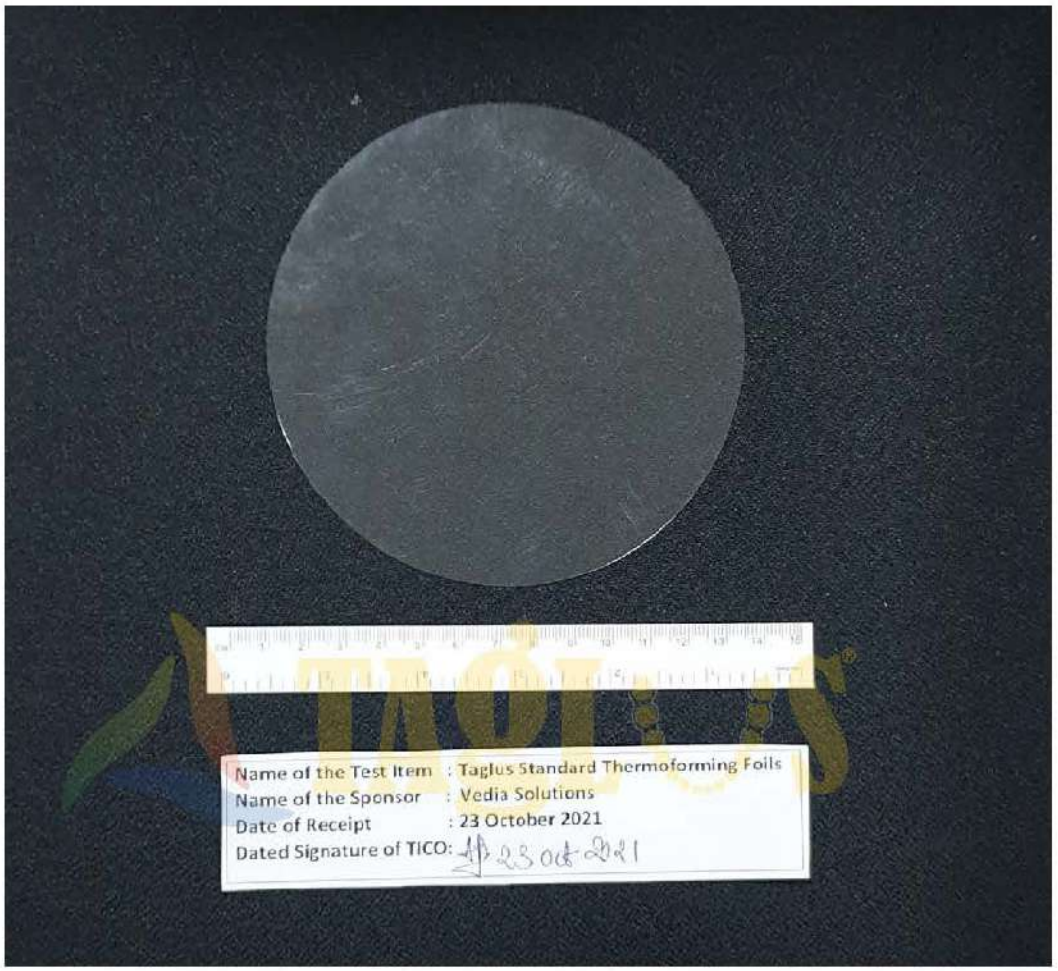
Total score = Sum of all the scores at test site (or) solvent control site;
Primary Irritation (PI) Score = Total score divided by 6;
Source: ISO 10993-23:2021(E)

Table 6: Calculation for Primary Irritation Index and Primary Irritation difference by using Primary Irritation Score

Animal number	1	2	3	PII*	PII difference#	4	5	6	PII*	PII difference#
Negative control site	0	0	0	0	0	0	0	0	0	0
Test item site	0	0	0	0		0	0	0	0	

* Primary irritation index (sum of all primary irritation scores/number of animals)
# PII difference = PII of test site - PII of control site
Source: ISO 10993-23:2021(E)

PHOTOGRAPH OF THE TEST ITEM



APPENDIX I

CONCISE POSITIVE CONTROL STUDY DATA

Study number	000/054
Study title	Skin Irritation Test in New Zealand White Rabbits
Study start date	27 September 2021
Experiment start date	28 September 2021
Experiment completion date	15 October 2021
Study completion date	20 October 2021

INTRODUCTION

Skin irritation is a key toxicity endpoint to assess biocompatibility of chemicals/medical devices. An assessment is made for testing the potential of the material under test to produce dermal irritation in rabbits following topical application.

OBJECTIVE

This skin irritation test was conducted to demonstrate the positive response of Sodium Lauryl Sulphate in New Zealand White Rabbits in compliance with ISO 10993-10:2010(E), ISO 10993-23:2021(E) and OECD 404:2015 guidelines.

DETAILS OF TEST ITEM [Sodium Lauryl Sulphate]

Appearance/Colour	Form: Powder, Colour: White
Manufacturer	Sigma Aldrich
CAS No.	151-21-3
Batch No.	0000009635
Manufacture Date	Not available
Expiry Date	August 2022
Concentration used in study	20% w/v

METHODOLOGY

This test was performed based on ISO 10993-10:2010(E), ISO 10993-23:2021(E) and OECD 404:2015 guidelines.

Two grams of Sodium Lauryl Sulphate was dissolved in distilled water and made up to 10 mL to obtain 20% w/v Sodium Lauryl Sulphate solution. Three male rabbits were clipped free of fur on dorsal side from an area of approximately 10 cm x 15 cm on both sides of the spinal cord approximately 16 h and 30 min prior to commencement of the experiment. The test item (0.5 mL) was applied onto the gauze measuring 6.25 cm^2 (2.5 cm x 2.5 cm) and placed on the test site in the dorsal region on the left cranial end and right caudal end

of rabbit skin. Similarly, 0.5 mL of the negative control (distilled water) was applied onto the gauze measuring 6.25 cm^2 (2.5 cm x 2.5 cm) and placed in the right cranial end and left caudal end on the control site.

The application sites were covered with a gauze patch (Make.: The Ramaraju Surgical Cotton Mills Limited; Batch No: 578/19; Expiry Date: July 2022) which was loosely held in contact with the skin by means of a suitable semi-occlusive dressing and non-irritant adhesive tape (Make.: 3M India Limited; Batch No.: R05190315; Expiry Date: April 2024) for all the animals. The patches were removed, 4 hours after the test item application and the test sites were marked with non-irritant permanent ink. No residues of the test item were found at the test site after patch removal.

STUDY RESULTS

Individual grades of skin reactions

	Observation Time (h)	Individual score																	
		Animal number 1						Animal number 2						Animal number 3					
		T_1	T_2	T	C_1	C_2	C	T_1	T_2	T	C_1	C_2	C	T_1	T_2	T	C_1	C_2	C
Erythema and Eschar formation	1	1	1	2	0	0	0	0	1	1	0	0	0	0	1	1	0	0	0
	24	1	2	3	0	0	0	2	2	4	0	0	0	2	2	4	0	0	0
	48	1	2	3	0	0	0	2	2	4	0	0	0	1	2	3	0	0	0
	72	2	2	4	0	0	0	2	2	4	0	0	0	2	2	4	0	0	0
	Day 7	1	0	1	0	0	0	1	1	2	0	0	0	0	0	0	0	0	0
Oedema formation	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	24	1	2	3	0	0	0	1	2	3	0	0	0	1	2	3	0	0	0
	48	1	1	2	0	0	0	1	2	3	0	0	0	2	1	3	0	0	0
	72	1	2	3	0	0	0	1	2	3	0	0	0	2	1	3	0	0	0
	Day 7	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0

C_1-First control site; C_2-Second control site; C-Sum of C_1 & C_2
T_1- First test site; T_2- Second test site; T-Sum of T_1 & T_2
Source: ISO 10993-10:2010 (E) and ISO 10993-23:2021(E)

Calculation of primary irritation score at three time points

Sites	Skin Reaction	Observation Time (h)	Individual score								
			Animal number 1			Animal number 2			Animal number 3		
			Score	Total Score	PI Score	Score	Total Score	PI Score	Score	Total Score	PI Score
Test (T)	Erythema and Eschar formation	24	3	18	3.0	4	21	3.5	4	20	3.3
		48	3			4			3		
		72	4			4			4		
	Oedema formation	24	3			3			3		
		48	2			3			3		
		72	3			3			3		
Control (C)	Erythema and Eschar formation	24	0	0	0	0	0	0	0	0	0
		48	0			0			0		
		72	0			0			0		
	Oedema formation	24	0			0			0		
		48	0			0			0		
		72	0			0			0		

Total score = Sum of all the scores at test site (or) negative control site;
Primary Irritation (PI) Score = Total score divided by 6;
Source: ISO 10993-10:2010 (E) and ISO 10993-23:2021(E)

Calculation for Primary Irritation Index and Primary Irritation difference by using Primary Irritation Score

Animal number	1	2	3	PII	PII difference
Negative control site	0	0	0	0	3.3
Test item site	3.0	3.5	3.3	3.3	

Primary irritation index (PII) = Sum of all primary irritation scores divided by 3
PII difference = PII of test site - PII of negative control site
Source: ISO 10993-10:2010 (E) and ISO 10993-23:2021(E)

DISCUSSION

Based on the primary irritation index obtained, 20% w/v Sodium Lauryl Sulphate is considered as an irritant to rabbit skin. Given that the mucosal membranes are more prone to irritant effects of chemicals, than the skin, it can be considered that 20% Sodium Lauryl Sulphate may induce irritation in mucosal membranes. Therefore, no separate animal experiments were performed in view of 3R's principles of animal testing.

CONCLUSION

Based on the results obtained, 20% w/v Sodium Lauryl Sulphate induced a primary irritation score of 3.3 and hence concluded as a moderate irritant under the conditions of the present study.

Summary of Positive Control Trial (GLR Study number 000/054)

Study number	Study start date	Experiment start date	Experiment completion date	Study completion date	Agent used	Result
000/054	27 September 2021	28 September 2021	15 October 2021	20 October 2021	20% Sodium Lauryl Sulphate	Moderate irritant

APPENDIX 2

Primary Irritation Index (PII)

Mean Score	Response category
0 to 0.4	Negligible
0.5 to 1.9	Slight
2 to 4.9	Moderate
5 to 8	Severe

Source: ISO 10993-23:2021(E)

RESPONSIBLE PERSONNEL

Mr. K. Sakthivel, MSc (Biotech)	Study Director
Ms. D. Nandhini, BPharm	Study Scientist
Ms. K. Vivekapriya, MPharm	Study Scientist
Dr. D. Yogaraj, MVSc	Study Scientist
Dr. S. Kavirajan, MVSc	Veterinarian
Dr. L. Mayavan, BVSc & AH	Animal House In-charge

STATEMENT OF STUDY COMPLIANCE

The study was performed in compliance with:

- OECD Principles of Good Laboratory Practice (revised 1997, issued January 1998) ENV/MC/CHEM (98) 17.
- US Food and Drug Administration's GLP regulations, 21 CFR Part 58 (subparts B to G and J)
- ISO/IEC 17025: 2017(E) (general requirements for the competence of testing and calibration laboratories).

All procedures were performed in accordance with GLR Laboratories Private Limited standard operating procedures (SOPs). The study was subjected to Quality Assurance evaluation by the GLR Laboratories Private Limited Quality Assurance Unit (QAU) in accordance with SOPs.

STUDY PLAN AMENDMENT

No study plan amendment was made during the conduct of the study.

STUDY PLAN DEVIATION

No study plan deviation occurred during the conduct of the study.

ARCHIVE STATEMENT

All primary data, or authenticated copies thereof, a sample test item, study plan and the final report will be retained for a period of 9 years in the GLR Laboratories Private Limited archives after issue of the final report. At the end of the specified archive period the Sponsor will be contacted to determine whether the data should be returned, retained or destroyed on their behalf. Sponsors will be notified of the financial implications of each of these options at that time.

DISTRIBUTION OF REPORTS

Two originals of the study report are prepared and distributed as mentioned below:

1. Sponsor.
2. Archive (GLR Laboratories Private Limited).

ANNEXURE 1

सत्यमेव जयते

GOVERNMENT OF INDIA
Department of Science and Technology
National Good Laboratory Practice (GLP) Compliance Monitoring Authority (NGCMA)

Certificate of GLP Compliance

This is to certify that

GLR Laboratories Private Limited
444, Gokulam Street, Mathur
Madhavaram, Chennai-600068 (Tamil Nadu), India

is a GLP certified test facility in compliance with the NGCMA's Document No. GLP-101 "Terms & Conditions of NGCMA for obtaining and maintaining GLP certification by a test facility" and OECD Principles of GLP.

The test facility conducts the below-mentioned tests/ studies:

- **Toxicity Studies**
- **Mutagenicity Studies**

The specific areas of expertise, test items and test systems are listed in the annexure overleaf.

Validity: March 13, 2020 – April 3, 2022

Certificate No. : GLP/C-132A/2020
Issue Date : 13-03-2020

(Dr. Neeraj Sharma)
Head, NGCMA