FINAL REPORT

Intracutaneous Reactivity Test of Taglus PU Flex Thermoforming Foils in New Zealand White Rabbits as per ISO 10993-23:2021

STUDY CONTRACT PARTNER:
UL India Private Limited
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UL Project Number: 4790342013

TEST FACILITY:
GLR Laboratories Private Limited,
444 Gokulam Street, Mathur, Chennai - 600 068, Tamil Nadu, India.
Study No.: 073/462

STUDY SPONSOR AND APPLICANT:
Vedia Solutions Div. of Laxmidental Export Pvt. Ltd.
103, Akruti arcade, J P Road,
Opp A H Wadhia School,
Andheri (W), Mumbai 400053

REPORT ISSUED DATE: 02 May 2022

FINAL REPORT

PRODUCT NAME:

Taglus PU Flex Thermoforming Foils

STUDY TITLE

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STUDY DIRECTOR AUTHENTICATION STATEMENT

Study No. : **073/462**

Study Title : **Intracutaneous Reactivity Test of Taglus PU Flex Thermoforming Foils in New Zealand White Rabbits as per ISO 10993-23:2021**

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).

Ms. N. Narmadha MSc, MPhil Study Director GLR Laboratories Private Limited	02 may 2022 Study Completion Date

* The identity and composition of the test item are the responsibility of the sponsor.

QUALITY ASSURANCE STATEMENT

Study No. : 073/462

Study Title : Intracutaneous Reactivity Test of Taglus PU Flex Thermoforming Foils in New Zealand White Rabbits as per ISO 10993-23:2021

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 of Inspection	Date of Inspection	Phase(s) of Study Inspected	Date of Reporting to Management, Study Director (Inspection No.)
1	Study Plan Verification	11 March 2022	Draft Study Plan	11 March 2022 (SBI/073/462/001)
2	Study Plan Verification	21 March 2022	Definitive Study Plan	21 March 2022 (SBI/073/462/002)
3	In-life Phase Inspection	02 April 2022	Test Item Extract Administration	02 April 2022 (SBI/073/462/003)
4	In-life Phase Inspection	05 April 2022	Grading of Skin Reactions	05 April 2022 (SBI/073/462/004)
5	Report Audit	27 April 2022	Draft Report	27 April 2022 (SBI/073/462/005)
6	Report Audit	02 May 2022	Final Report	02 May 2022 (SBI/073/462/006)

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.

N. Parthiban

02 MAY 2022

Dr. Parthiban Natarajan, PhD, ERT
Head-Quality Assurance
GLR Laboratories Private Limited

Date

TEST FACILITY MANAGEMENT STATEMENT

Study No. : **073/462**

Study Title : **Intracutaneous Reactivity Test of Taglus PU Flex Thermoforming Foils in New Zealand White Rabbits as per ISO 10993-23:2021**

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.

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

02 May 2023
Date

SUMMARY

Intracutaneous reactivity potential of Taglus PU Flex Thermoforming Foils, supplied by Vedia Solutions Div. of Laxmidental Export Pvt. Ltd. was evaluated in female (nulliparous and non-pregnant) New Zealand white rabbits.

The test item, Taglus PU Flex Thermoforming Foils is a transparent sheet with a diameter, 12.5 cm and thickness, 0.08 cm. It is a surface device which comes in contact with mucosal membrane. The duration of contact is less than 24 hours (limited). According to ISO 10993-1:2018, this is a surface device which comes in contact with mucosal membrane and the duration of contact is up to 24 hours (limited).

The test item was extracted at a ratio of 6 cm^2/mL (since the thickness of test item is less than 0.5 mm) in polar solvent (physiological saline) as well as non-polar solvent (cottonseed oil) at 37 ± 1 °C for 72 ± 2 h and 05 minutes. The total surface area of the test item is measuring approximately 441 cm^2 (as calculated in our laboratory). Polar extract was prepared by extracting one test item in 73.5 mL of physiological saline. Similarly, the non-polar extract was prepared by extracting one test item in 73.5 mL of cottonseed oil under sterile conditions. Solvent controls were also maintained at 37 ± 1 ºC for 72 h and 05 minutes. This fulfils the requirement of ISO 10993-12:2012 and ISO 10993-12:2021.

At the end of extraction, extracts and solvent controls were clear without any colour change or particulates. No changes were observed in the retrieved test item. No additional processing such as filtration, centrifugation, pH adjustments or any other processing were made. The extracts and solvent controls were transferred to sterile containers and were used within 1 h and 55 minutes of preparation and were considered stable during this time.

About 15 h and 40 minutes prior to intracutaneous injections, fur on all the rabbits were closely clipped off their backs, allowing sufficient distance on both sides of the spine for injection of test item.

The extracts and solvent controls were injected intracutaneously (0.2 mL/injection/test sites) into the rabbits. Animals were observed immediately after injection, and at 24, 48, and 72 h for morbidity, mortality and abnormal clinical signs and symptoms. The skin reactions were visually scored according to ISO 10993-23:2021, at 24 h, 48 h and 72 h post injection.

No mortality or morbidity was observed in the experimental animals. A gradual increase in body weight was observed during the study period. No signs of ill health were observed in any of the animals. Animals treated with the test item extracts did not show any skin

reactions. The difference of the mean skin reaction scores for the test item extracts and the solvent control was zero.

Positive control trials for intracutaneous reactivity test are carried out periodically at GLR Laboratories Private Limited using sodium lauryl sulphate as the positive control, to comply with the requirements of ISO 10993-23:2021. The most recent positive control study completed on 01 February 2022 within the test facility indicated a clear positive result (Mean reaction score of 7.6) and the negative control used in the study gave a mean irritation score of 0. Therefore, the assay was considered valid.

Based upon the results obtained and in line with ISO 10993-23:2021, the given test item, Taglus PU Flex Thermoforming Foils, supplied by Vedia Solutions Div. of Laxmidental Export Pvt. Ltd., is considered non-reactive in New Zealand white rabbits.

INTRODUCTION

Biocompatibility testing is a regulatory requirement for demonstrating the safety of medical devices. The general guidance for biocompatibility testing is given in ISO 10993-1:2018, Biological Evaluation of Medical Devices - Part 1, Evaluation and Testing within a Risk Management Process. The primary aim of this group of standards is the protection of humans from potential biological risks arising from the use of medical devices. This standard also describes the categorization of medical devices based on nature and duration of patient contact; and test selection necessary to evaluate biocompatibility. The technical guidance for the biocompatibility tests are given in other parts of ISO 10993.

Intracutaneous reactivity test is carried out according to ISO 10993-23:2021. Types of irritation tests are listed below:

Irritation Tests	**Standard**
Animal Irritation Test Animal intracutaneous (intradermal) reactivity test	ISO 10993: Part 23:2021
Special irritation tests	
Ocular irritation test Oral mucosa irritation test Penile irritation test Rectal irritation test Vaginal irritation test	ISO 10993: Part 23:2021

The reactivity potential of a test item is assessed by injecting the test item intracutaneously in rabbits and the responses are graded as given in ISO 10993-23:2021.

OBJECTIVE

To determine the reactivity potential of the test item following intracutaneous injection in New Zealand white rabbits.

STUDY DATES

Study Start Date	21 March 2022
Experiment Start Date	26 March 2022
Experiment Completion Date	05 April 2022

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

TEST ITEM DETAILS

The test item, Taglus PU Flex Thermoforming Foils was received at GLR Laboratories Private Limited on 02 March 2022 and stored at 20.1 to 24.3 °C until use.

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

TEST ITEM DETAILS

Test Item	Taglus PU Flex Thermoforming Foils
Batch / Lot No.	22022010-01
Manufacture Date	02 February 2022
Expiry Date	02 February 2025
Appearance	Transparent sheet
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 intracutaneous reactivity are carried out periodically at GLR Laboratories Private Limited in New Zealand White rabbits using sodium lauryl sulphate in compliance with ISO 10993-23:2021. The trial completed on 01 February 2022 gave a clear positive reaction (Appendix 1).
Solvent controls	<u>Physiological saline (0.9% w/v sodium chloride solution)</u>
	Manufacturer: Fresenius kabi India pvt. Ltd.
	Batch No.: 93QE204001
	Expiry Date: April 2024
	Appearance: Colourless clear liquid

Cottonseed oil	
Manufacturer	Sigma-Aldrich
Lot No.	MKCM9272
Expiry Date	October 2026
Appearance	Yellow viscous liquid

Determinations of stability and characteristics of the test item were the responsibility of the sponsor. The test item was handled with necessary protective clothing and all recommended safety and sterile measures were followed.

Description of the test item

The test item, Taglus PU Flex Thermoforming Foils is a transparent sheet with a diameter, 12.5 cm and thickness, 0.08 cm. It is a surface device which comes in contact with mucosal membrane. The duration of contact is less than 24 hours (limited). According to ISO 10993-1:2018, this is a surface device which comes in contact with mucosal membrane and the duration of contact is up to 24 hours (limited).

TEST SYSTEM

Species	*Oryctolagus cuniculus* (rabbit)
Strain	New Zealand White
Weight range (g) (at the time of dosing)	2288.9 to 2378.2
Sex	Female (nulliparous and non-pregnant)
Source	M/s. VAB Biosciences #1-6-197/45/D, Bapuji Nagar Musheerabad, Hyderabad-500020, India. This supplier approved by the committee for the purpose of control and supervision of experiments on animals (CPCSEA) for sale of experimental animals
Quarantine period	7 days
Number of animals used	3
Acclimation period	7 days
Justification for animal use	Intracutaneous injection test in rabbits is specified in the current ISO testing standards and has been used historically to evaluate biomaterials.

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

ANIMAL HUSBANDRY

Test room no.	22
Test room temperature (°C)	18.0 to 21.9
Relative humidity (%)	37 to 59
Housing	Animals were housed individually in standard rabbit cages.
Method of identification	Animals were identified using cage cards indicating cage number, study number, species, strain, animal number, sex, body weight, dose and individual earmarking with individual numbers.
Feed	Commercial rabbit pellet feed manufactured by M/s. VRK Nutritional Solutions D-47 & W-38, MIDC area, Miraj, Dist. Sangli- 416410, India.
Water	Purified drinking water was provided *ad libitum*
Bedding material	No bedding material will be used as rabbits are housed in stainless steel cages with mesh floors. A tray with sterilized corn cob will be used to collect the excreta and urine and will be changed every day. This will not have direct contact with rabbits. Corn cob supplied by: M/s. Matha Agrotech Plot No. R-15, SY.No.847/3, KSSIDC Industrial Estate 2nd Stage, Ranebennur – 581115 India
Photoperiod	12: 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

The test item was extracted at a ratio of 6 cm^2/mL (since the thickness of test item is less than 0.5 mm) in polar solvent (physiological saline) as well as non-polar solvent (cottonseed oil) respectively at 37 ± 1 °C for 72 ± 2 h and 05 minutes. This fulfils the requirement of ISO 10993-12:2012 and ISO 10993-12:2021.

The details of extracts preparation are as follows:

Solvent/Extract	Extraction vehicle	Surface area of the test item	Volume of vehicle (mL)	Extract preparation start time	Extract preparation end time	Condition of extracts
Polar Extract	Physiological saline	441	73.5	09:00 am on 30 Mar 2022	09:05 am on 02 Apr 2022	Colourless clear solution; no particulates*
Polar Solvent Control	Physiological saline	NA	10			Colourless clear solution; no particulates
Non-polar Extract	Cottonseed oil	441	73.5			Yellow viscous liquid; no particulates*
Non-polar Solvent Control	Cottonseed oil	NA	10			Yellow viscous liquid; no particulates

NA-Not applicable; Extraction duration 72 h and 05 minutes.
* No change in appearance of the extraction solvent after extraction.

No additional processing such as filtration, centrifugation, pH adjustments or any other processing were made. No changes were observed in the retrieved test item. The extracts and solvent controls were transferred to sterile containers and used within 1 h and 55 minutes of preparation. The preparations were considered stable during this time.

Dosing Procedure

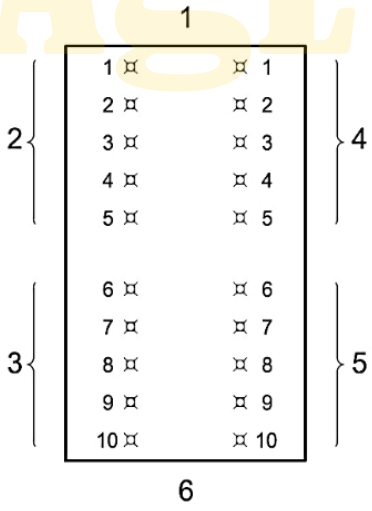
Justification	Intracutaneous injection of test item extracts to rabbit are recommended as a suitable route of administration in ISO 10993-23:2021 to determine biocompatibility of materials used in medical devices.

Test procedure

The pH of the polar extract was 7.02. Therefore, the extract was found suitable to conduct intracutaneous reactivity study in rabbits. The pH of the oil extract cannot be measured, but it is assumed acceptable for intracutaneous injections.

About 15 h and 40 minutes prior to intracutaneous injections, fur on all the rabbits were closely clipped off their backs, allowing sufficient distance on both sides of the spine for injection (diagram below). Intracutaneous injections of polar and non-polar extracts and corresponding controls were given using, 1 mL sterile syringes and needles (Hindustan Syringes & Medical Devices Ltd.; Batch No.:049013AG32; Expiry date: November 2025) as given in the table and figure:

Animal No.	Sample	Injection site	Volume of each injection (mL)	No. of injections/ site
1	Test item extract in physiological saline Physiological saline (solvent control) Test item extract in cottonseed oil Cottonseed oil (solvent control)	Left cranial end Right cranial end Left caudal end Right caudal end	0.2	5
2	Test item extract in physiological saline Physiological saline (solvent control) Test item extract in cottonseed oil Cottonseed oil (solvent control)	Left cranial end Right cranial end Left caudal end Right caudal end	0.2	5
3	Test item extract in physiological saline Physiological saline (solvent control) Test item extract in cottonseed oil Cottonseed oil (solvent control)	Left cranial end Right cranial end Left caudal end Right caudal end	0.2	5



1. Cranial end; 2. 0.2 ml injections of polar extract; 3. 0.2 ml injections of non-polar extract; 4. 0.2 ml injections of polar solvent control; 5. 0.2 ml injections of non-polar solvent control; 6. Caudal end
Source: ISO 10993- Part 23: 2021

OBSERVATIONS

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

Body weight
Body weight of each animal was recorded prior to dosing and end of the experiment.

Clinical observation
Animals were observed for clinical signs of toxicity immediately after intracutaneous injection, and at 24 h, 48 h, and 72 h.

Scoring of skin reaction
Skin reactions viz., oedema, erythema and eschar formation were scored visually with naked eyes as per ISO 10993-23:2021 at 24 h, 48 h and 72 h following the intracutaneous injection. Observations were graded on a numerical scale for both the test item and negative control.

Grading system for intracutaneous reactions are shown in the following table:

Reaction	Numerical grading
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 extending beyond exposure area)	4
Maximal possible score for irritation	8

Source: ISO 10993- Part 23: 2021

Euthanasia
Animals were euthanized by Thiopental sodium injection at the end of the experiment.

Necropsy and gross pathology
Necropsy and gross pathology were not performed.

EVALUATION CRITERIA

After 72 h grading, all erythema and oedema grades at 24 h, 48 h and 72 h were totalled for each test item extract and control for each individual animal. For calculating the score of a test item and control on each individual animal, the derived value was divided by 15 (3 scoring periods x 5 test or control sample injection sites). To determine the overall mean score for each test item and each corresponding control, the scores for the 3 animals were added and divided by three. The final test item score was obtained by subtracting the score of the control from the test item score.

Solvent controls	Mean Reaction Score for test item extract	Mean Reaction Score for solvent control	Overall difference (Test extract - control)
Physiological saline	A	B	(A-B)
Cottonseed oil	C	D	(C-D)

If the difference between the mean reaction grades (erythema/ oedema) for the test item and the control is greater than 1.0, then the test item was considered to cause intracutaneous reactivity.

RESULTS

Mortality and morbidity

No mortality or morbidity occurred in any of the experimental animals.

Body weight

Increase in body weight were observed in all the animals (Table 1).

Clinical observation

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

Scoring of skin reaction

Injected sites appeared normal immediately after the injections. Grading of skin reactions for individual animals are given in Tables 2 and 3. The difference of the mean skin reaction scores for the test item extracts and the solvent control was zero (Table 4).

Positive control trial

Positive control trial conducted within the test facility gave clear positive results (Appendix 1).

CONCLUSION

Based upon the results obtained and in line with ISO 10993-23:2021, the given test item, Taglus PU Flex Thermoforming Foils, supplied by Vedia Solutions Div. of Laxmidental Export Pvt. Ltd., is considered non-reactive in New Zealand white rabbits.

REFERENCES

1. ISO 10993-1:2018. Biological evaluation of medical devices - Part 1: Evaluation and testing within a risk management process.
2. ISO 10993-2:2006. Biological evaluation of medical devices - Part 2: Animal welfare requirements.
3. ISO 10993-23:2021. Biological evaluation of medical devices - Part 23: Tests for irritation.
4. ISO 10993-12:2012. Biological evaluation of medical devices - Part 12: Sample preparation and reference materials.
5. ISO 10993-12:2021. Biological evaluation of medical devices - Part 12: Sample preparation and reference materials.
6. ISO 1705:2018: Dentistry - Evaluation of biocompatibility of medical devices used in dentistry.
7. 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.
8. ISO/IEC 17025: 2017. General requirements for the competence of testing and calibration laboratories.
9. 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 of New Zealand white rabbits

Animal No.	Sex	Body weight (g)	
		Initial	Final
1	F	2327.3	2333.4
2		2378.2	2383.7
3		2288.9	2294.8

F-Female

Table 2: Grading of skin reactions for individual New Zealand white rabbits

Time points			24 h				48 h				72 h			
Animal No.	Sex	Sites	Test item Extract in polar solvent		Polar Solvent control		Test item Extract in polar solvent		Polar Solvent control		Test item Extract in polar solvent		Polar Solvent control	
			E	O	E	O	E	O	E	O	E	O	E	O
1	Female	1	0	0	0	0	0	0	0	0	0	0	0	0
		2	0	0	0	0	0	0	0	0	0	0	0	0
		3	0	0	0	0	0	0	0	0	0	0	0	0
		4	0	0	0	0	0	0	0	0	0	0	0	0
		5	0	0	0	0	0	0	0	0	0	0	0	0
		E+O	0		0		0		0		0		0	
		E+O/ 5 sites	0		0		0		0		0		0	
2	Female	1	0	0	0	0	0	0	0	0	0	0	0	0
		2	0	0	0	0	0	0	0	0	0	0	0	0
		3	0	0	0	0	0	0	0	0	0	0	0	0
		4	0	0	0	0	0	0	0	0	0	0	0	0
		5	0	0	0	0	0	0	0	0	0	0	0	0
		E+O	0		0		0		0		0		0	
		E+O/ 5 sites	0		0		0		0		0		0	
3		1	0	0	0	0	0	0	0	0	0	0	0	0
		2	0	0	0	0	0	0	0	0	0	0	0	0
		3	0	0	0	0	0	0	0	0	0	0	0	0
		4	0	0	0	0	0	0	0	0	0	0	0	0
		5	0	0	0	0	0	0	0	0	0	0	0	0
		E+O	0		0		0		0		0		0	
		E+O/ 5 sites	0		0		0		0		0		0	

E, Erythema; O, Oedema

Table 3: Grading of skin reactions for individual New Zealand white rabbits

Time points			24 h				48 h				72 h			
Animal No.	Sex	Sites	Test item Extract in Non-polar solvent		Non-polar solvent control		Test item Extract in Non-polar solvent		Non-polar solvent control		Test item Extract in Non-polar solvent		Non-polar solvent control	
			E	O	E	O	E	O	E	O	E	O	E	O
1	Female	1	0	0	0	0	0	0	0	0	0	0	0	0
		2	0	0	0	0	0	0	0	0	0	0	0	0
		3	0	0	0	0	0	0	0	0	0	0	0	0
		4	0	0	0	0	0	0	0	0	0	0	0	0
		5	0	0	0	0	0	0	0	0	0	0	0	0
		E+O	0		0		0		0		0		0	
		E+O/ 5 sites	0		0		0		0		0		0	
2		1	0	0	0	0	0	0	0	0	0	0	0	0
		2	0	0	0	0	0	0	0	0	0	0	0	0
		3	0	0	0	0	0	0	0	0	0	0	0	0
		4	0	0	0	0	0	0	0	0	0	0	0	0
		5	0	0	0	0	0	0	0	0	0	0	0	0
		E+O	0		0		0		0		0		0	
		E+O/ 5 sites	0		0		0		0		0		0	
3		1	0	0	0	0	0	0	0	0	0	0	0	0
		2	0	0	0	0	0	0	0	0	0	0	0	0
		3	0	0	0	0	0	0	0	0	0	0	0	0
		4	0	0	0	0	0	0	0	0	0	0	0	0
		5	0	0	0	0	0	0	0	0	0	0	0	0
		E+O	0		0		0		0		0		0	
		E+O/ 5 sites	0		0		0		0		0		0	

E, Erythema; O, Oedema

Table 4: Calculation of skin reactions - Overall mean score and difference

Animal No.	Individual animal score [(E+O)/5 sites] / 3 time points			
	Test item extract in polar solvent	Polar solvent	Test item extract in non-polar solvent	Non-Polar solvent
1	0	0	0	0
2	0	0	0	0
3	0	0	0	0
Overall mean score	0	0	0	0
Overall difference	0		0	

Overall difference = Overall mean score of test item - Solvent control

E, Erythema; O, Oedema

PHOTOGRAPH OF THE TEST ITEM

APPENDIX I

CONCISE POSITIVE CONTROL STUDY DATA

Study Number	000/003-21-POS
Study Title	Intracutaneous reactivity test in New Zealand White Rabbits
Study Start Date	27 December 2021
Experiment Start Date	03 January 2022
Experiment Completion Date	13 January 2022
Study Completion Date	01 February 2022

INTRODUCTION

Animal irritation test by intracutaneous (intradermal) administration is a key toxicity endpoint to assess the irritation potential of chemicals/medical devices. An assessment is made for testing the potential of the material under test to produce intracutaneous reactivity in rabbits following the administration (intracutaneous) of test item.

OBJECTIVE

This intracutaneous reactivity test was conducted to demonstrate the positive response of Sodium Lauryl Sulphate in New Zealand White Rabbits in order to confirm the sensitivity and reliability of the test performed at GLR Laboratories Private Limited in compliance with ISO 10993-23:2021 standards.

DETAILS OF POSITIVE CONTROL ITEM [Sodium lauryl sulphate]

Appearance/Colour	Form: powder, Colour: White
Manufacturer	Sigma Aldrich
Batch No.	0000009635
Manufacture Date	Not available
Expiry Date	August 2022
Concentration used in study	0.1% w/v sodium lauryl sulphate

METHODOLOGY

This test was performed based on ISO 10993-23:2021.

Sodium lauryl sulphate (0.01 gram) was dissolved in sterile water for injection and made up to 10 mL to obtain 0.1% 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 (0.2 mL) was injected (intracutaneous) at five sites on one side of each rabbit using sterile syringe and needles. Similarly, solvent control (sterile water for injection) was injected at five sites on the contralateral side of each rabbit.

Animal No.	Sample	Injection site	Volume of each injection (mL)	No. of injections/ site
1	Test item in sterile water for injection (0.1 %)	Left cranial end	0.2	5
	Sterile water for injection	Right cranial end		
2	Test item in sterile water for injection (0.1 %)	Left cranial end	0.2	5
	Sterile water for injection	Right cranial end		
3	Test item in sterile water for injection (0.1 %)	Left cranial end	0.2	5
	Sterile water for injection	Right cranial end		

Source: ISO 10993: Part 23: 2021

STUDY RESULTS

Grading of skin reactions for individual New Zealand white rabbits

Animal No.	24 h				48 h				72 h			
	Test item		Control item		Test item		Control item		Test item		Control item	
	E	O	E	O	E	O	E	O	E	O	E	O
1	4	4	0	0	4	4	0	0	4	3	0	0
	3	4	0	0	3	4	0	0	4	4	0	0
	4	3	0	0	4	4	0	0	4	4	0	0
	4	4	0	0	4	3	0	0	3	3	0	0
	4	4	0	0	4	4	0	0	4	4	0	0
2	4	4	0	0	4	4	0	0	3	4	0	0
	4	4	0	0	4	4	0	0	4	4	0	0
	4	3	0	0	3	4	0	0	4	3	0	0
	4	4	0	0	4	4	0	0	4	3	0	0
	4	4	0	0	4	4	0	0	4	4	0	0
3	4	4	0	0	3	3	0	0	4	3	0	0
	4	4	0	0	3	4	0	0	4	4	0	0
	3	4	0	0	4	4	0	0	4	3	0	0
	4	4	0	0	4	4	0	0	3	4	0	0
	4	4	0	0	4	3	0	0	4	4	0	0

E, Erythema; O, Oedema

Total Irritation scores

Animal Number	Test item (Total Score/15)	Negative Control (Total Score/15)
1	113/15=7.5	0
2	115/15=7.7	0
3	112/15=7.5	0

Mean reaction scores

Solvent	Test item	Negative Control	Overall difference
Sterile water for injection	7.6	0	7.6

E, Erythema; O, Oedema

DISCUSSION

The skin reactions were visually scored according ISO 10993-23:2021 at 24 h, 48 h and 72 h, post injection. The animals were observed for three consecutive days for morbidity, mortality and abnormal clinical signs and symptoms following injections. Neither mortality nor morbidity and no signs of ill health were observed. However, skin reactions were observed in all test sites. Test item injected sites showed erythema and oedema at 24 h, 48 h and 72 h and no reactions were observed at the control sites.

At 24 h observation, at all test site necrosis and discoloration of skin and around the test site a severe erythema was observed. In addition, a severe oedema was observed in all the test sites which was raised more than 2 mm. The gradings of skin reactions were provided in the table. A gradual increase in the body weight was observed in test and control animals.

CONCLUSION

Based on the results obtained, 0.1% w/v sodium lauryl sulphate induced a mean reaction of 7.6 score. The difference between the mean reaction scores (erythema/ oedema) for the test and the control is greater than 1.0, henceforth 0.1% w/v sodium lauryl sulphate is considered to cause intracutaneous reactivity under the conditions of the present study. This confirms the sensitivity and reliability of the test performed at GLR Laboratories Private Limited.

Summary of positive control trial (GLR Study number 000/003-21-POS)

Study number	Study start date	Experiment start date	Experiment completion date	Study completion date	Agent used	Result
000/003-21-POS	27 December 2021	03 January 2022	13 January 2022	01 February 2022	0.1% Sodium Lauryl Sulphate	Reactive

RESPONSIBLE PERSONNEL

Ms. N. Narmadha, MSc, MPhil	Study Director
Dr. C. Aiswarya, Pharm-D	Study Scientist
Dr. S. Sherni Prithiba Jhansirani, Pharm-D	Study Scientist
Dr. S. Kavirajan, MVSc	Veterinarian
Dr.L. Mayavan BVSc &AH	Animal House In-charge

STATEMENT OF STUDY COMPLIANCE

This 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 (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, tissue specimens, blocks and slides (if applicable), the study plan with its amendments (if any) and the final report will be retained for a period of 9 years after issue of the final report in the archives of GLR Laboratories

Private Limited. The archived sample of test item will be retained for 2 years beyond its date of expiry. At the end of the archival period the study sponsor will be contacted to determine whether the archived contents should be either retained for a further period, returned to the sponsor, or destroyed by GLR Laboratories as per in-house standard operating procedure in compliance with the principles of GLP. Sponsors will be notified of the financial implications, if any, 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

भारत सरकार ★ Govt. of India
राष्ट्रीय जी एल पी अनुपालन निगरानी प्राधिकरण
National GLP Compliance Monitoring Authority
विज्ञान एवं प्रौद्योगिकी विभाग ★ Dept. of Science and Technology

(Dr. Neeraj Sharma)
Head, NGCMA

ANNEXURE 2

GLR Laboratories Pvt Ltd
committed to scientific & service excellence

Declaration of

Compliance to Principles of Good Laboratory Practice and GLP Certification status of GLR Laboratories

This is to declare that there is no change in the status of GLP certification of GLR Laboratories Private Limited.

The present 'Certification of GLP Compliance' of GLR Laboratories (Certificate Number: GLP/C-132A/2020) is valid up to 03 April 2022. In compliance with the "Terms and Conditions of NGCMA for Obtaining and Maintaining GLP Certification by a Test Facility" (Document No.: GLP-101; Issue No.: 08; Issue Date: October 25, 2019) of the National GLP Compliance Monitoring Authority (NGCMA) of India (Department of Science and Technology, Government of India), GLR Laboratories has successfully completed the recertification inspection by the NGCMA during the dates 26 to 28 Mar 2022, well within the present tenure of certification. The renewed GLP compliance certificate of GLR Laboratories, based on the inspection and action taken report, will be issued by the NGCMA from the present validity period of 03 April 2022 extending up to the next three-year period, i.e., 02 April 2025, without any break in the tenure of GLP certification.

30 MAR 2022

(Dr. Parthiban Natarajan)
Head Quality Assurance & Assistant Director
GLR Laboratories Pvt Ltd.

30 Mar 2022

(Dr. S. S. Murugan)
Test Facility Management
GLR Laboratories Pvt Ltd.

Date: 30 Mar 2022

OECD-GLP | ISO/IEC 17025 | Drug Controller Approved Laboratory

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