FINAL REPORT

Skin Sensitization Test in Guinea Pigs (Guinea Pig Maximization Test) of Taglus PU FLEX Thermoforming Foils as per ISO 10993-10:2021(E).

STUDY CONTRACT PARTNER:
UL India Private Limited
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UL Project Number: 4790186870

TEST FACILITY:
GLR Laboratories Private Limited,
444, Gokulam Street, Mathur, Chennai - 600 068, Tamil Nadu, India.
Study No.: 073/436

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

REPORT ISSUED DATE: 09 February 2022

FINAL REPORT

PRODUCT NAME:

Taglus PU FLEX Thermoforming Foils

STUDY TITLE

Skin Sensitization Test in Guinea Pigs (Guinea Pig Maximization Test) of Taglus PU FLEX Thermoforming Foils as per ISO 10993-10:2021(E)

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STUDY DIRECTOR AUTHENTICATION STATEMENT

Study No : **073/436**

Study Title : **Skin Sensitization Test in Guinea Pigs (Guinea Pig Maximization Test) of Taglus PU FLEX Thermoforming Foils as per ISO 10993-10:2021(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).

09 Feb 2022

Dr. D. Yogaraj, MVSc
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 : 073/436

Study Title : Skin Sensitization Test in Guinea Pigs (Guinea Pig Maximization Test) of Taglus PU FLEX Thermoforming Foils as per ISO 10993-10:2021(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 of Inspection	Date of Inspection	Phase(s) of Study Inspected	Date of Reporting to Management, Study Director (Inspection No.)
1	Study Plan Verification	20 November 2021	Draft Study Plan	20 November 2021 (SBI/073/436/001)
2	Study Plan Verification	25 November 2021	Definitive Study Plan	25 November 2021 (SBI/073/436/002)
3	In Life Phase Inspection	13 December 2021	Test Item Extract Administration - Intradermal Phase	13 December 2021 (SBI/073/436/003)
4	In Life Phase Inspection	20 December 2021	Test Item Extract Application - Topical Phase	20 December 2021 (SBI/073/436/004)
5	In Life Phase Inspection	03 January 2022	Test Item Extract Application - Challenge Phase	03 January 2022 (SBI/073/436/005)
6	In Life Phase Inspection	06 January 2022	Grading of Skin Reactions	06 January 2022 (SBI/073/436/006)

S. No.	Type of Inspection	Date of Inspection	Phase(s) of Study Inspected	Date of Reporting to Management, Study Director (Inspection No.)
7	Report Audit	20 January 2022	Draft Report	20 January 2022 (SBI/073/436/007)
8	Report Audit	09 February 2022	Final Report	09 February 2022 (SBI/073/436/008)

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. Parth — 09 FEB 2022

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

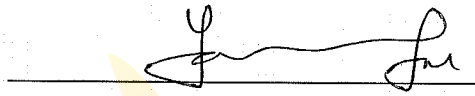
Date

TEST FACILITY MANAGEMENT STATEMENT

Study No : **073/436**

Study Title : **Skin Sensitization Test in Guinea Pigs (Guinea Pig Maximization Test) of Taglus PU FLEX Thermoforming Foils as per ISO 10993-10:2021(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.



09 Feb 2022

Ms. M. Yaminy, B.Com, (MBA)
Deputy Test Facility Management
GLR Laboratories Private Limited

Date

SUMMARY

Skin sensitization potential of Taglus PU FLEX Thermoforming Foils supplied by Vedia Solutions was evaluated in male guinea pigs using guinea pig maximization test (GPMT).

The test item, Taglus PU FLEX Thermoforming Foils is a transparent disk with a diameter of 12.5 cm and thickness less than 0.5 mm. It is a surface device which comes in contact with mucosal membrane. The duration of contact is less than 24 hours (limited).

The test item was extracted at a ratio of 6 cm^2/ mL (since the thickness of the 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 (intradermal induction - 71 h and 50 min, topical application - 72 h and 16 min & challenge phase - 72 h and 03 min) under sterile conditions. The total surface area of the test item 111 cm^2 (as calculated in our laboratory). For each intradermal induction phase, topical induction phase and challenge phase, polar extract was prepared by extracting 111 cm^2 (01 no.) of test item in 18.5 mL of physiological saline and non-polar extract was prepared by extracting 111 cm^2 (01 no.) of test item in 18.5 mL of cottonseed oil. Solvent controls were also subjected to the similar extraction conditions. This fulfils the requirement of ISO 10993-12:2012(E) and ISO 10993-12:2021(E).

At the end of extraction, both extracts and solvent controls were clear without any colour change or particulates. No changes were observed in the retrieved test item. No additional processing's such as filtration, centrifugation, pH adjustments or any other processing were made. The extracts and solvent controls were transferred to sterile containers and used as such within a maximum of 02 h and 05 min of preparation and were considered stable during this time.

Animals were divided into four groups; G1 - five guinea pigs for polar solvent control, G2 - ten guinea pigs for polar test item extract, G3 - five guinea pigs for non-polar solvent control and G4 - ten guinea pigs for non-polar test item extract. The fur over the treatment sites were clipped and shaved on the day of treatment, prior to dosing on all the animals. Induction of sensitization was a two-stage procedure with intradermal injections on day 0 (with Freund's complete Adjuvant (FCA), solvent and extracts), followed by a topical patch exposure on day 7 for 48 h. On day 21, challenge patches were applied for 24 h. Skin reaction grading was performed using Magnusson and Kligman scale at 24 h and 48 h, after removing the challenge patches according to ISO 10993-10:2021(E).

Positive control trials for sensitization are carried out periodically at GLR Laboratories Private Limited in guinea pigs using 2,4-Dinitrochlorobenzene in compliance with regulatory guidelines. The trial completed on 19 October 2021 gave a clear sensitizing reaction in all (100%) treated animals. No response was observed in solvent controls treated animals. Therefore, the assay was considered valid.

No mortality or morbidity was observed in any of the animals used in this study. A gradual increase in body weight was observed in all the animals at the end of the experiment. No skin sensitization reactions were observed in both control and test sites of the animals. Therefore, no gross and histopathological examination were conducted.

Based upon the results obtained in this study and in line with ISO 10993-10:2021(E), the given test item, Taglus PU FLEX Thermoforming Foils, supplied by Vedia Solutions, is considered as a non-sensitizer to Guinea Pigs 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.

Sensitization (Type IV hypersensitivity reaction) is a key toxicity endpoint to assess the biocompatibility of medical devices. Guinea pig maximization test is a sensitive method to determine the sensitization potential of medical devices, both in terms of induction and elicitation.

OBJECTIVE

To determine the skin sensitization potential of the test item using guinea pig maximization test (GPMT).

STUDY DATES

Study Start Date	25 November 2021
Experiment Start Date	06 December 2021
Experiment Completion Date	06 January 2022

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 PU FLEX Thermoforming Foils, was received at GLR Laboratories Private Limited, on 13 November 2021 and stored at 20.1 to 24.1 °C until use.

The following test item information provided by the sponsor were considered an adequate description of the characterisation, purity and stability of the test item.

Test Item	Taglus PU FLEX Thermoforming Foils
Batch \ Lot No.	12029092-1
Manufacture Date	29 September 2021
Expiry Date	20 September 2024
Appearance	Transparent disk
Ingredients	PU (PolyUrethane)

Temperature Stability	37 °C
Sterility	Non-Sterile
Positive Control	2,4-Dinitrochlorobenzene
	Positive control trials for sensitization are carried out periodically at GLR Laboratories Private Limited in guinea pigs using 2,4-Dinitrochlorobenzene in compliance with the regulatory guidelines. The trial completed on 19 October 2021 gave a clear sensitizing reaction in all (100%) treated animals (Appendix 3).
Solvent controls	Physiological saline (0.9% w/v Sodium Chloride solution) Manufacturer: Eurolife Healthcare Private Limited Batch No.: 10210671B Expiry date: Sep 2024 Appearance: Colourless clear liquid Cottonseed oil Manufacturer: Sigma-Aldrich Lot No.: MKCM9272 Expiry Date: Oct 2026 Appearance: Yellow coloured viscous liquid

The test item was handled with necessary protective clothing and all recommended safety and sterile measures were followed. Determinations of stability and characteristics of the test item were 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 PU FLEX Thermoforming Foils is a transparent disk with a diameter of 12.5 cm and thickness less than 0.5 mm. It is a surface device which comes in contact with mucosal membrane. The duration of contact is less than 24 hours (limited).

TEST SYSTEM

Species	*Cavia porcellus* (Guinea pig)
Strain	Dunkin – Hartley
Weight range (g) (at the time of dosing)	319.84 to 399.63
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	30
Number of groups	4
Number of animals per group	Physiological saline control: 5 Physiological saline extract: 10 Cottonseed oil control: 5 Cottonseed oil extract: 10
Acclimatization period	7 days
Justification for animal use	Guinea pigs are selected because there is a large volume of background data on this species. Recommended in ISO 10993, Part-10:2021(E) standard as an appropriate species to evaluate skin sensitization 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.	09
Test room temperature (°C)	18.0 to 21.9
Relative humidity (%)	37 to 58
Housing	Animals were housed individually in polypropylene cages.
Method of identification	Animals were identified using cage cards indicating cage no., study no., species, strain, group no., animal no., sex, body weight and dose.
Feed	Commercial Guinea pig pellet feed. Supplier - VRK Nutritional Solutions D-47 & W-38, MIDC area, Miraj, Dist Sangli- 416410, Maharashtra (India).
Water	Purified drinking water supplemented with vitamin C was provided *ad libitum*.
Bedding material	Sterilized paddy husk

	Supplier - M/S K. Dhandapani 4/331, Old Mahabalipuram Road Kottivakkam, Chennai-600041 Tamilnadu (India).
Photoperiod	12 h light and 12 h dark cycle
Contaminants	Contaminants, reasonably expected in feed and/or water supplied were 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	Only healthy young adults, previously unused animals 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 the 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 under sterile conditions. The total surface area of the test item is 111 cm^2 (as calculated in our laboratory). Solvent controls were also subjected to same extraction conditions. This fulfils the requirement of ISO 10993-12:2012(E) and ISO 10993-12:2021(E).

Day 0: Intradermal induction phase

The required volume of extract was prepared freshly prior to dosing as follows:

Solvent/Extract	Extraction vehicle	Surface area of the test item (cm^2) *	Volume of vehicle (mL)	Extract preparation start time	Extract preparation end time	Condition of extracts
Polar Solvent Control	Physiological saline	NA	10	10:10 am on 10 Dec 2021	10:00 am on 13 Dec 2021	Colourless clear solution; no particulates
Polar Extract	Physiological saline	111	18.5			Colourless clear solution; no particulates
Non-polar Solvent Control	Cottonseed oil	NA	10			Yellow viscous liquid; no particulates
Non-polar Extract	Cottonseed oil	111	18.5			Yellow viscous liquid; no particulates

*01 no. of test item was used for extraction; Extraction duration: 71 h and 50 min; NA-Not applicable.

No changes were observed in 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 stored at room temperature. All extracts and solvent controls were used within 1 h and 16 min of preparation and were considered stable during this time.

Day 7: Topical application

The required volume of extract was prepared freshly prior to dosing as follows:

Solvent/Extract	Extraction vehicle	Surface area of the test item (cm^2) *	Volume of vehicle (mL)	Extract preparation start time	Extract preparation end time	Condition of extracts
Polar Solvent Control	Physiological saline	NA	10	09:46 am on 17 Dec 2021	10:02 am on 20 Dec 2021	Colourless clear solution; no particulates
Polar Extract	Physiological saline	111	18.5			Colourless clear solution; no particulates
Non-polar Solvent Control	Cottonseed oil	NA	10			Yellow viscous liquid; no particulates
Non-polar Extract	Cottonseed oil	111	18.5			Yellow viscous liquid; no particulates

*01 no. of test item was used for extraction; Extraction duration: 72 h and 16 min; NA-Not applicable.

No changes were observed in 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 stored at room temperature. All extracts and solvent controls were used within 2 h and 05 min of preparation and were considered stable during this time.

Day 21: Challenge phase

The required volume of extract was prepared freshly prior to dosing as follows:

Solvent/Extract	Extraction vehicle	Surface area of the test item (cm^2) *	Volume of vehicle (mL)	Extract preparation start time	Extract preparation end time	Condition of extracts
Polar Solvent Control	Physiological saline	NA	10	10:17 am on 31 Dec 2021	10:20 am on 03 Jan 2022	Colourless clear solution; no particulates
Polar Extract	Physiological saline	111	18.5			Colourless clear solution; no particulates
Non-polar Solvent Control	Cottonseed oil	NA	10			Yellow viscous liquid; no particulates

Non-polar Extract	Cottonseed oil	111	18.5		Yellow viscous liquid; no particulates

*01 no. of test item was used for extraction; Extraction duration: 72 h and 03 min; NA-Not applicable.

No changes were observed in 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 stored at room temperature. All extracts and solvent controls were used within 49 min of preparation and were considered stable during this time.

Test Procedure

Justification for method of administration	The method of administration is in line with the ISO 10993-10:2021(E) standard. For the induction phase, intradermal injections and the topical application were employed. The challenge phase was accomplished by topical applications.

Animals were divided into four groups; G1 - five guinea pigs for polar solvent control, G2 - ten guinea pigs for polar test item extract, G3 - five guinea pigs for non-polar solvent control and G4 - ten guinea pigs for non-polar test item extract.

The fur over the treatment sites in all animals were clipped and shaved on the day of treatment, prior to dosing. Induction of sensitization was a two-stage procedure with initial intradermal injections, followed by a topical patch exposure on day 7.

Intradermal Induction phase

On day 0, 0.1 mL intradermal injections (1 mL syringe, make: Hindustan Syringes & Medical Devices Ltd, Batch No. 049013AG32, Expiry Date: November 2025) of the test item extracts, solvents and Freund's Complete Adjuvant (FCA) (Sigma-Aldrich; Lot No. SLBZ9885; Expiry date: May 2024) in various mixtures were administered to the solvent control and test groups (Appendix 1).

Control group:

Site A: 1: 1 mixture (v/v) Freund's Complete Adjuvant + solvent (solution A)
Site B: Polar solvent or non-polar solvent (solution B)
Site C: 1: 1 mixture of solution A and solution B

Test group:

Site A: 1: 1 mixture (v/v) Freund's Complete Adjuvant + solvent (solution A)
Site B: Polar extract or non-polar extract of test item (solution B)
Site C: 1: 1 mixture of solution A and solution B

Topical Induction Phase

Since no irritation was observed following the intradermal injections, on day 6, the test area was treated with 10% Sodium Lauryl Sulphate (Avantor Performance Material India Limited; Batch No.: J159K18; Expiry date: November 2023) in petroleum jelly (Make: HiMedia Laboratories Pvt Ltd; Lot No. 0000314448; Expiry Date: November 2022).

On day 7, absorbent gauze patch (The Ramaraju Surgical Cotton Mills Limited; Batch No.: 578/19; Expiry date: July 2022) measuring 8 cm^2 loaded with 0.5 mL of test item extracts and solvents, respectively was placed topically to respective groups of guinea pigs, on the same site as that of intradermal injections. The over patch was covered loosely with an occlusive dressing which was held in place for 48 h.

Challenge phase

On day 21, the challenge exposure was administered as a topical patch. Absorbent gauze patch measuring 8 cm^2 loaded with 0.5 mL of test item extract was placed on the left side and the patch with solvent control was placed on the right side of each animal in respective groups for 24 h at sites other than those used for intradermal injections/topical applications and the application sites were marked with a non-irritant permanent marker ink. The details of the experiment are summarized in Appendix 1.

OBSERVATIONS

Mortality & Morbidity

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

Body weight

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

Grading of skin reactions

Grading of skin reactions was performed visually at 24 and 48 h after removing the challenge patch. The challenge application sites were assessed for erythema and edema using Magnusson and Kligman scale (Appendix 2).

Euthanasia

Animals were euthanized by carbon dioxide (CO_2) exposure at the end of the experiment.

Necropsy and Gross pathology

Since no abnormality was observed in any animals, necropsy and gross pathology were not performed.

DATA EVALUATION

A comparison of the biological responses seen following skin sensitization of the test item extracts and solvents were reported and interpreted, using good scientific judgement.

Skin reactions elicited in terms of incidence and severity of reactions, scored as per the Magnusson and Kligman grading scale, between the test item extracts treated and solvent control groups were compared.

ACCEPTANCE CRITERIA

The study is considered valid, since the following criteria are met:

1. Positive control trial conducted within the test facility gave clear positive results.
2. No response was observed in solvent control treated animals.

RESULTS

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

Body weight
A gradual increase in the body weight was observed in all the animals at the end of the experiment. Body weight of animals recorded prior to dosing and end of the experiment are given in Table 1.

Grading of skin reactions
Grading of skin reactions performed at 24 h and 48 h after removing the challenge patch are given in Table 2. No sensitization reactions were observed in animals treated with the solvent controls. No evidence of sensitization was seen in any of the test item treated animals, as no skin reactions were observed.

Histopathology
No gross and histopathological examination were found necessary in this study.

CONCLUSION

Based upon the results obtained in this study and in line with ISO 10993-10:2021(E), the given test item, Taglus PU FLEX Thermoforming Foils, supplied by Vedia Solutions, is considered as a non-sensitizer to Guinea Pigs 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 10, Tests for Skin Sensitization, ISO 10993-10:2021(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, ISO 10993 - Part 1. Evaluation and Testing Within a Risk Management Process. Guidance for Industry and Food and Drug Administration Staff. September 4, 2020.

PHOTOGRAPH OF THE TEST ITEM

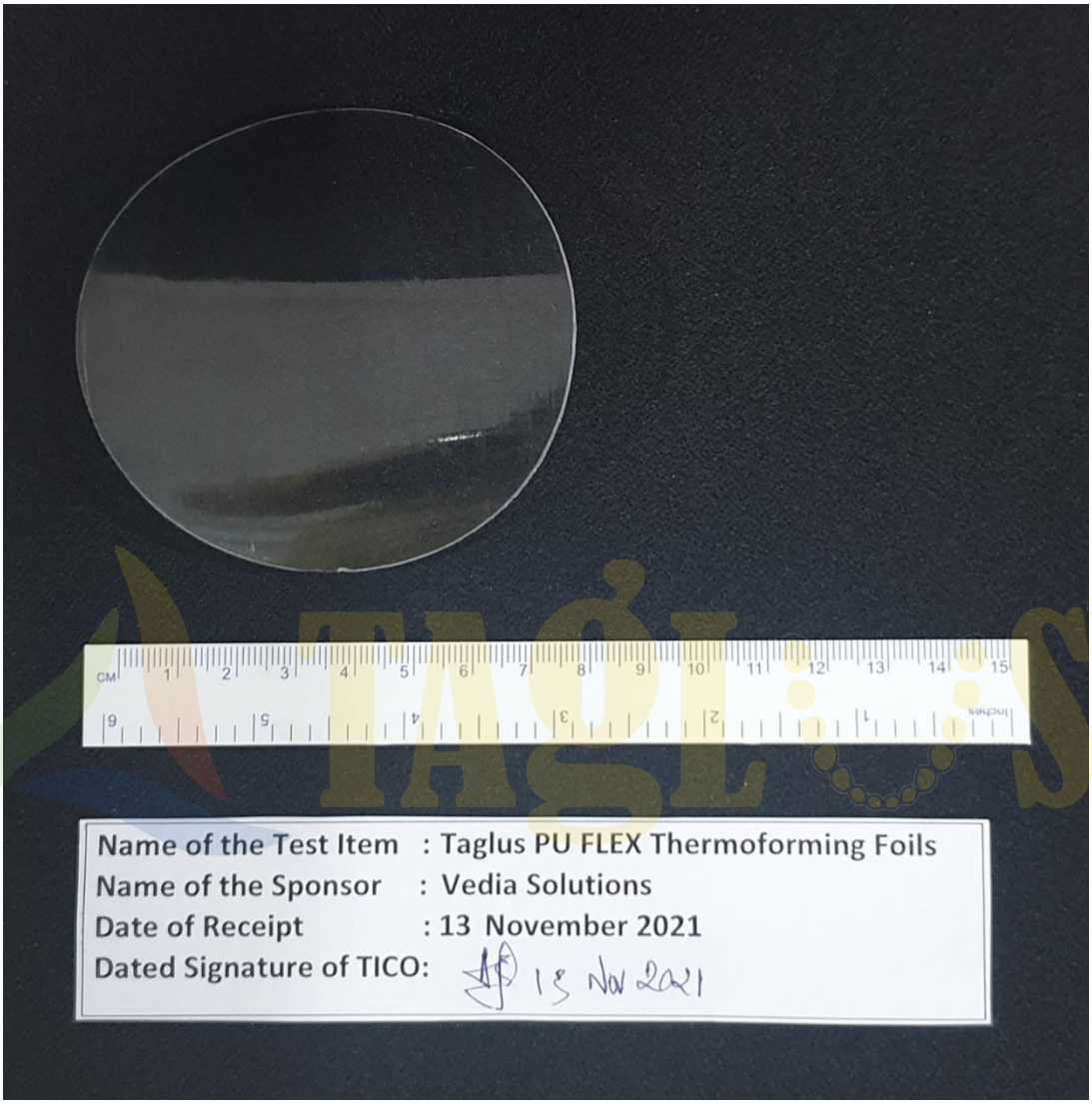


Table 1: Individual body weights of all animals

Group	Sex	Animal No.	Weight (in grams)		
			At the time of dosing	At the end of experiment	Increase in weight
G1	M	01	384.01	422.54	38.53
		02	391.32	427.74	36.42
		03	322.51	362.10	39.59
		04	360.99	391.19	30.20
		05	320.05	359.51	39.46
		Mean ± SD	**355.78 ± 33.43**	**392.62 ± 32.24**	**36.84 ± 3.92**
G2	M	06	383.35	422.28	38.93
		07	399.63	439.58	39.95
		08	369.19	400.59	31.40
		09	334.32	372.82	38.50
		10	337.46	370.75	33.29
		11	390.48	422.61	32.13
		12	319.84	352.63	32.79
		13	330.55	365.89	35.34
		14	381.77	415.96	34.19
		15	375.56	406.95	31.39
		Mean ± SD	**362.22 ± 28.76**	**397.01 ± 29.42**	**34.79 ± 3.24**
G3	M	16	397.30	429.04	31.74
		17	350.62	386.73	36.11
		18	328.02	367.42	39.40
		19	333.13	363.28	30.15
		20	389.79	427.62	37.83
		Mean ± SD	**359.77 ± 32.06**	**394.82 ± 31.85**	**35.05 ± 3.96**
G4	M	21	380.00	410.01	30.01
		22	391.33	430.86	39.53
		23	369.18	406.06	36.88
		24	370.10	404.93	34.83
		25	338.66	369.06	30.40
		26	340.11	379.19	39.08
		27	342.34	376.59	34.25
		28	330.63	361.39	30.76
		29	365.32	395.41	30.09
		30	369.90	406.75	36.85
		Mean ± SD	**359.76 ± 20.33**	**394.03 ± 21.74**	**34.27 ± 3.76**

M- Male; SD- Standard Deviation

Table 2: Grading of skin reaction after removal of challenge patch

Group	Sex	Animal No.	Magnusson and Kligman Scale			
			24 h		48 h	
			C	T	C	T
G1	M	01	0	0	0	0
		02	0	0	0	0
		03	0	0	0	0
		04	0	0	0	0
		05	0	0	0	0
G2	M	06	0	0	0	0
		07	0	0	0	0
		08	0	0	0	0
		09	0	0	0	0
		10	0	0	0	0
		11	0	0	0	0
		12	0	0	0	0
		13	0	0	0	0
		14	0	0	0	0
		15	0	0	0	0
G3	M	16	0	0	0	0
		17	0	0	0	0
		18	0	0	0	0
		19	0	0	0	0
		20	0	0	0	0
G4	M	21	0	0	0	0
		22	0	0	0	0
		23	0	0	0	0
		24	0	0	0	0
		25	0	0	0	0
		26	0	0	0	0
		27	0	0	0	0
		28	0	0	0	0
		29	0	0	0	0
		30	0	0	0	0

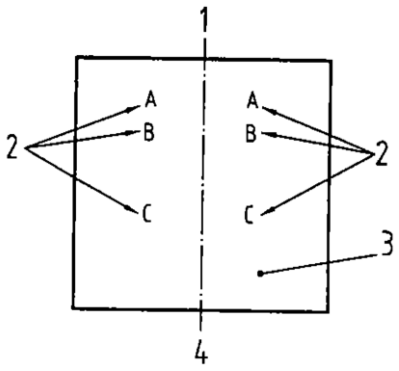
M-Male; C- Control site; T- Treated site; h- hour

APPENDIX 1

Test Procedure

Group	Animal No.	Sex	Treatment Group	Intradermal Induction Phase (0.1 mL)			Topical induction phase (0.5 mL per patch) *		Challenge phase # (0.5 mL per patch) *
				Injection I (Solution A)	Injection II (Solution B)	Injection III (Solution C)	10% SLS	Treatment	
G1	1-5	M	Polar solvent control	1: 1 mixture of FCA and polar solvent	Polar solvent	1: 1 mixture of sol. A and sol. B	Yes	Polar solvent	Polar solvent & Polar extract of Test item
G2	6-15	M	Test item in polar solvent	1: 1 mixture of FCA and polar solvent	Polar extract of test item	1: 1 mixture of sol. A and sol. B	Yes	Polar extract of Test item	Polar solvent & Polar extract of Test item
G3	16-20	M	Non-polar solvent control	1: 1 mixture of FCA and non-polar solvent	Non-polar solvent	1: 1 mixture of sol. A and sol. B	Yes	Non polar solvent	Non polar solvent & Non polar extract of Test item
G4	21-30	M	Test item in non-polar solvent	1: 1 mixture of FCA and non-polar solvent	Non-polar extract of test item	1: 1 mixture of sol. A and sol. B	Yes	Non-polar extract of Test item	Non-polar solvent & Non-polar extract of Test item

M- Male; FCA - Freund's Complete Adjuvant; SLS-Sodium Lauryl Sulphate;
* Gauze patch size = 8 cm^2 approximately
# Two challenge patches are placed on upper flank, one on left side and other on right side
Intradermal Injection was given on Day 0 at sites A, B and C
Topical application was applied on Day 7
Challenge dose was applied on Day 20
Sites A, B and C are shown below:



1 - Cranial end; 2 - 0.1 ml intradermal injection sites; 3 - Clipped intrascapular region; 4 - Caudal end
Source: ISO 10993-10:2021(E).

APPENDIX 2

Magnusson and Kligman scale

Patch test reaction	Grading scale
No visible change	0
Discrete or patchy erythema	1
Moderate and confluent erythema	2
Intense erythema and/or swelling	3

Source: ISO 10993-10:2021(E).

APPENDIX 3

CONCISE POSITIVE CONTROL STUDY DATA

Study Number	000/053
Study Title	Skin Sensitization Test in Guinea Pigs (Guinea Pig Maximization Test)
Study Start Date	07 September 2021
Experiment Start Date	14 September 2021
Experiment Completion Date	14 October 2021
Study Completion Date	19 October 2021

OBJECTIVE

To ensure the reproducibility and sensitivity of the test procedure at the test facility, skin sensitization test with positive control 2,4-Dinitrochlorobenzene is performed in compliance with OECD 406, 1992 and ISO 10993-10:2010(E) standard.

CONTROL ITEM DETAILS [2,4-Dinitrochlorobenzene (DNCB)]

Manufacturer	Sigma-Aldrich
Appearance \ Colour	Crystalline \ Faint yellow
Batch No.	BCBS4201V
CAS No.	97-00-7
Molecular Formula	$C_6H_3ClN_2O_4$
Molecular Weight	202.55 g/mol
Date of Receipt	05 March 2018
Expiry date	04 March 2023

METHODOLOGY

This study was performed based on OECD 406, 1992 and ISO 10993-10:2010(E) standard.

Induction: Intradermal Injections

On day 0, 0.1 mL intradermal injection of the following were given to the fur clipped animals in the treated group. Site 1: a 1:1 mixture (v/v) of FCA & physiological saline (Solution A), site 2: a 0.025% w/v of DNCB in 1:4 v/v acetone: cottonseed oil (Solution B) and site 3: a 1:1 mixture of solution A and solution B.

Similarly, 0.1 mL intradermal injection of the following were given to the fur clipped animals in the control group. Site 1: a 1:1 mixture (v/v) of FCA & physiological saline (Solution A), site 2: a 1:4 v/v acetone: cottonseed oil (Solution B) and site 3: a 1:1 mixture of solution A and solution B.

Induction: Topical Application

Since DNCB induced skin reaction, Sodium Lauryl Sulphate was not applied on the day before the topical treatment. On day 7, the test and control area were again cleared of fur and an absorbent gauze patch (2 cm x 4 cm) loaded with 0.2 mL of 0.25 % w/v of DNCB in a 1:4 v/v acetone: cottonseed oil and 0.2 mL of 1:4 v/v acetone: cottonseed oil, was placed on respective groups. The patch was then held in contact by an occlusive dressing for 48 h.

Challenge: Topical Application

On day 21, the flanks of treated and control animals were cleared of fur. Absorbent gauze patch loaded with 0.2 mL of 0.1 % w/v of DNCB in 1:4 v/v acetone: cottonseed oil was applied on left side and absorbent gauze patch loaded with 0.2 mL of 1:4 v/v acetone: cottonseed oil was applied on right side of each animal in respective groups at the sites other than those used for intradermal injections/topical applications. The gauze patches were held in contact by an occlusive dressing for 24 h, then the patch was removed and the application sites were marked with non-irritant marker ink. At 21 h, after removing the patch, the challenge area was cleaned and closely-clipped. The application sites were scored at 24 h and 48 h after patch removal using a Magnusson and Kligman grading scale.

Magnusson and Kligman Grading Scale
(For evaluation of Challenge patch test reactions)

Patch test reaction	Grading scale
No visible change	0
Discrete or patchy erythema	1
Moderate and confluent erythema	2
Intense erythema and swelling	3

STUDY RESULTS

Grading of skin reaction after removal of the challenge patch

Group	Animal No.	Magnusson and Kligman Scale			
		At control site		At treated site	
		24 h	48 h	24 h	24 h
G1	1	0	0	0	0
	2	0	0	0	0
	3	0	0	0	0
	4	0	0	0	0
	5	0	0	0	0

G2	6	0	0	1	1
	7	0	0	2	1
	8	0	0	2	2
	9	0	0	2	2
	10	0	0	2	1
	11	0	0	1	1
	12	0	0	1	1
	13	0	0	2	1
	14	0	0	2	2
	15	0	0	2	1

CONCLUSION

The results indicated that animals treated with 2,4-Dinitrochlorobenzene (Batch No. BCBS4201V), induced sensitization reactions in 100% of treated animals. Therefore, according to OECD Guidelines for Testing of Chemicals, 406, 1992 and ISO 10993-10:2010 (E), 2,4-Dinitrochlorobenzene (Batch No. BCBS4201V) is categorized as a strong sensitizer under the conditions of the present study.

Summary of positive control trial for skin sensitization, GPMT (000/053)

Study start date	Experiment Start Date	Experiment Completion Date	Study Completion date	Concentration of 2,4-Dinitrochlorobenzene			Vehicle used	Result	
				Induction Phase 1 (Intradermal)	Induction Phase 2 (Topical)	Challenge Phase		No of animals +ve	Maximum reaction grading
07 September 2021	14 September 2021	14 October 2021	19 October 2021	0.025% w/v	0.25% w/v	0.1% w/v	1:4 v/v acetone: cottonseed oil	+ve in 10/10 animals	Grade 2 - Moderate and confluent erythema

RESPONSIBLE PERSONNEL

Dr. D. Yogaraj, MVSc	Study Director
Ms. K. Vivekapriya, MPharm	Study Scientist
Ms. D. Nandhini, BPharm	Study Scientist
Mr. K. Sakthivel, MSc (Biotech)	Study Scientist
Dr. K. 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(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 occurred during the conduct of this study.

STUDY PLAN DEVIATION

No study plan deviation occurred during the conduct of this 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

भारत सरकार ★ Govt. of India
National GLP Compliance Monitoring Authority
Dept. of Science and Technology

(Dr. Neeraj Sharma)
Head, NGCMA