FINAL REPORT

Skin Irritation Test in New Zealand White Rabbits of Nitrile Examination Glove - Powder Free as per ISO 10993-10:2010(E).

STUDY CONTRACT PARTNER:
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TEST FACILITY:
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444, Gokulam Street, Mathur, Chennai - 600 068, Tamil Nadu, India.
Study No.: 702/002

STUDY SPONSOR AND APPLICANT:
AT Glove Engineering Sdn. Bhd.
9, Jalan Chepor 11/7
Kawasan Perusahaan Seramik Chepor
31200 Chemor, Perak, Malaysia

FINAL REPORT

PRODUCT NAME:

Nitrile Examination Glove - Powder Free

STUDY TITLE

Skin Irritation Test in New Zealand White Rabbits of Nitrile Examination Glove - Powder Free as per ISO 10993-10:2010(E)

STUDY NUMBER
702/002

TEST FACILITY:
GLR Laboratories Private Limited
444, Gokulam Street
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Tamil Nadu, India

REPORT ISSUED DATE **13 January 2021**

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31200 Chemor, Perak, Malaysia.

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STUDY DIRECTOR AUTHENTICATION STATEMENT

Study No. : **702/002**

Study Title : **Skin Irritation Test in New Zealand White Rabbits of Nitrile Examination Glove - Powder Free as per ISO 10993-10:2010(E)**

This study was performed in accordance with the mutually agreed study plan, 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).

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

13 Jan 2021
Study Completion Date

* with the exception of the identity and composition of the test item, which was the responsibilities of the sponsor.

QUALITY ASSURANCE STATEMENT

Study No. : 702/002

Study Title : Skin Irritation Test in New Zealand White Rabbits of Nitrile Examination Glove - Powder Free as per ISO 10993-10:2010(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	31 October 2020	Draft Study Plan	31 October 2020 (SBI/702/002/001)
2	Study Plan Verification	23 November 2020	Definitive Study Plan	23 November 2020 (SBI/702/002/002)
3	In-life Phase Inspection	12 December 2020	Test Item Extracts Application	12 December 2020 (SBI/702/002/003)
4	In-life Phase Inspection	15 December 2020	Grading of Skin Reactions	15 December 2020 (SBI/702/002/004)
5	Report Audit	28 December 2020	Draft Report	28 December 2020 (SBI/702/002/005)
6	Report Audit	13 January 2021	Final Report	13 January 2021 (SBI/702/002/006)

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.

G. Velmani

13 JAN' 2021

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

Date

TEST FACILITY MANAGEMENT STATEMENT

Study No. : **702/002**

Study Title : **Skin Irritation Test in New Zealand White Rabbits of Nitrile Examination Glove - Powder Free as per ISO 10993-10:2010(E)**

This is to certify that, the Test Facility Management appointed and provided the Study Director 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

13 Jan 2021
Date

SUMMARY

Skin irritation potential of Nitrile Examination Glove - Powder Free, supplied by AT Glove Engineering Sdn. Bhd., was evaluated in male New Zealand White rabbits.

The test item, Nitrile Examination Glove - Powder Free is a surface device which comes in contact with skin. The dimensions of the test item are: length: 243 to 250 mm, thickness: palm: 0.11 to 0.14 mml finger: 0.14 to 0.17 mm (as stated by sponsor). The duration of contact (short-term) is less than 4 hours.

Sixteen hours and fifty minutes prior to the commencement of the experiment, fur on all the rabbits were gently clipped on the dorsal side for an area of approximately 10 cm x 15 cm on both sides of the spinal cord.

Test item measuring 6.25 cm^2 (2.5 cm x 2.5 cm) was cut and placed topically in the dorsal region on the left cranial end and right caudal end (test site) on each rabbit. A total of six rabbits (three animals for inner surface of the test item and three animals for outer surface of the test item) was used in this study. Similarly, absorbent gauze (negative control) measuring 6.25 cm^2 (2.5 cm x 2.5 cm) was cut and placed topically at the control sites of the fur clipped area of rabbit skin, in the dorsal region on the right cranial end and left caudal end of six rabbits.

The application sites were covered with an absorbent gauze patch measuring, 2.5 cm x 2.5 cm, and the patches were loosely held in contact with the skin by means of a suitable semi-occlusive dressing for the duration of 4 h. The test item and control item patches were removed at the end of 4 h exposure. No residues were found at the test site after patch removal.

The skin reactions were visually scored according to ISO 10993-10:2010(E) at 1, 24, 48 and 72 h following patch removal. The animals were observed for three consecutive days for morbidity, mortality and abnormal clinical signs and symptoms following the patch removal.

Positive control trials for irritation are carried out once in three months in GLR Laboratories Private Limited. The most recent positive control study was completed on 31 August 2020 which gave a “moderate irritant” reaction. No response was observed in solvent control treated animals. Therefore, the assay was considered valid. The next positive control trial has been initiated in November 2020.

No mortality or morbidity were observed in the experimental animals. An 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 was '0'. The results indicated that the animals treated with the test item (inner and outer surface of the glove) did not show any skin irritation.

Based upon the results obtained in this study and in line with ISO 10993-10:2010 (E) it is concluded that the test item, Nitrile Examination Glove - Powder Free, supplied by AT Glove Engineering Sdn. Bhd., is considered as non-irritant to New Zealand white rabbits.

INTRODUCTION

Biocompatibility testing is a regulatory requirement for demonstrating preclinical safety of medical devices. This is evaluated in line with the standard, 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	23 November 2020
Experiment Start Date	05 December 2020
Experiment Completion Date	15 December 2020

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

TEST ITEM DETAILS

The test item, Nitrile Examination Glove - Powder Free was received at GLR Laboratories Private Limited on 19 November 2020 and stored at room temperature (20 to 30 °C) until used.

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

Test Item	Nitrile Examination Glove - Powder Free
Batch No / Lot No.	200918000088
Manufacture Date	September 2019
Expiry Date	September 2023
Appearance	Blue powder free glove
Ingredients	Synthetic Polymer - Nitrile Polymer

Temperature Stability	70 °C
Sterility	Non - Sterile

CONTROL ITEM DETAILS

Positive Control	Sodium Lauryl Sulphate (SLS)
Manufacturer	Sigma Aldrich
Batch No.	0000009635
Expiry date	August 2022
	Positive control trials for irritation are conducted once in three months in GLR laboratories Private Limited. The trial completed on 31 August 2020 gave a "moderate irritant" reaction (Appendix 1). The next positive control trial has been initiated in November 2020.
Negative Control	Physiological saline

Manufacturer	Eurolife Healthcare Pvt. Ltd.
Batch No.	10190939B
Expiry Date	August 2022
Appearance	Colourless clear solution

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, Nitrile Examination Glove - Powder Free is a surface device which comes in contact with skin. The dimensions of the test item are: length: 243 to 250 mm, thickness: palm: 0.11 to 0.14 mml finger: 0.14 to 0.17 mm (as stated by sponsor). The duration of contact (short-term) is less than 4 hours.

TEST SYSTEM

Species	*Oryctolagus cuniculus* (Rabbit)
Strain	New Zealand White
Weight range (g) (at the time of dosing)	2136.1 to 2589.0
Sex	Male

Source	VAB Bio Sciences, Hyderabad, India. This supplier is approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India for breeding laboratory animals.
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, Part-10:2010 (E) standard as an appropriate species to evaluate skin irritation of medical devices and recommended 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.	03
Test room temperature (°C)	19.2 to 22.0
Relative humidity (%)	37 to 60
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, signature and individual ear marking.
Feed	Commercial rabbit pellet feed (VRK Nutritional)
Water	Purified drinking water was provided *ad libitum*
Bedding material	No bedding materials were used as rabbits were housed in stainless steel cages with mesh floors. A tray with sterilized paddy husk were used to collect excreta and urine, were changed daily. They have not direct contact with any animals.
Photoperiod	12 h: 12 h light and dark cycle
Contaminants	Contaminants, reasonably expected in feed and/or water supplied were not believed to influence the outcome of the study.

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

Test item measuring 6.25 cm^2 (2.5 cm x 2.5 cm) was cut and placed topically in the dorsal region on the left cranial end and right caudal end (test site) on each rabbit. A total of six rabbits (three animals for inner surface of the test item and three animals for outer surface of the test item) was used in this study. Similarly, absorbent gauze (negative control) measuring 6.25 cm^2 (2.5 cm x 2.5 cm) was cut and placed topically at the control sites of the fur clipped area of rabbit skin, in the dorsal region on the right cranial end and left caudal end (control site) of six rabbits.

Dosing procedure

Justification for method of application	Recommended in ISO 10993, Part-10:2010 (E) standard, dermal application of test item is recommended as a suitable method of administration to determine skin irritation in evaluating the biocompatibility of medical devices.

Test procedure

The animals with healthy intact skin were selected for this study. Sixteen hours and fifty minutes prior to commencement of the study, fur on all the rabbits were clipped free on the dorsal side for an area of approximately 10 cm x 15 cm on either side of the vertebral column.

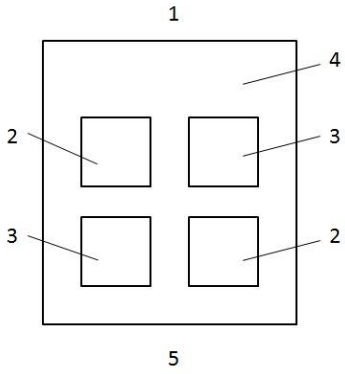
Topical Application

Test item measuring 6.25 cm^2 (2.5 cm x 2.5 cm) was cut and the inner surface of the glove was placed as such on the fur clipped area of rabbit skin, in the dorsal region on the left cranial end and right caudal end. The right cranial end and left caudal end was treated with an absorbent gauze (Make: The Ramaraju Surgical Cotton Mill Ltd., Batch No: 578/19; Expiry: July 2022) (negative control) measuring approximately 6.25 cm^2 (2.5 cm x 2.5 cm) as shown in the figure.

Similarly, test item measuring 6.25 cm^2 (2.5 cm x 2.5 cm) was cut and the outer surface of the glove was placed as such on the fur clipped area of rabbit skin, in the dorsal region on the left cranial end and right caudal end. The right cranial end and left caudal end was

treated with an absorbent gauze (negative control) measuring approximately 6.25 cm^2 (2.5 cm x 2.5 cm).

The application sites were covered with a gauze patch measuring 2.5 cm x 2.5 cm and the patches were 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: April 2024) for the duration of 4 h (to reflect cumulative duration of contact between the glove and user's skin). The treatment sites were marked with non-irritant permanent marker ink. The test item and control item patches were removed at the end of 4 h. No residues were found at the test site after patch removal.



1. Cranial end; 2. Test site; 3. Control site; 4. Clipped dorsal region; 5. Caudal end.
Source: ISO 10993: Part 10: 2010 (E)

OBSERVATIONS

Mortality & Morbidity

Animals were observed daily for mortality and morbidity for a period of three days, following the patch removal.

Body Weight

Body weight of each animal were recorded prior to dosing and at end of the 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. Dermal 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 10993-10:2010 (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 10993-10:2010 (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.

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 all the following criteria are met:

1. Positive control trial conducted within the test facility indicated a clear positive result.
2. Negative control used in the study gave a mean irritation score of 0 to 0.4.

RESULTS

Mortality & Morbidity
No mortality and morbidity were observed in any of the animals used in this study.

Body Weight
An increase in body weight at the end of the experiment was observed. Individual body weight of the animals is given in Table 1.

Clinical Observations
No signs of ill health or overt toxicity were observed.

Grading of skin reactions
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 & Table 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 Table 4, 5 & 6. The observations were not extended to 14 days since no skin lesions were found. Histopathological examination was not performed since unequivocal responses were observed.

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

Necropsy & Gross pathology
None of the animals were found dead or in moribund condition and therefore, no gross pathology was conducted.

CONCLUSION

Based upon the results obtained in this study and in line with ISO 10993-10:2010 (E) it is concluded that the test item, Nitrile Examination Glove - Powder Free, supplied by AT Glove Engineering Sdn. Bhd., is considered as non-irritant to New Zealand white rabbits.

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 Irritation and Skin Sensitization, ISO 10993-10:2010(E).

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

5. 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.

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

7. 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 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	2589.0	2612.2
2		2136.1	2163.1
3		2491.2	2515.6
4		2537.3	2560.6
5		2193.5	2218.4
6		2555.8	2578.3

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 10993-10:2010(E) Clause 6.3.6

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 10993-10:2010(E) Clause 6.3.6

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) negative control site;
Primary Irritation (PI) Score = Total score divided by 6;
Source: ISO 10993-10:2010(E) Clause 6.3.6

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		

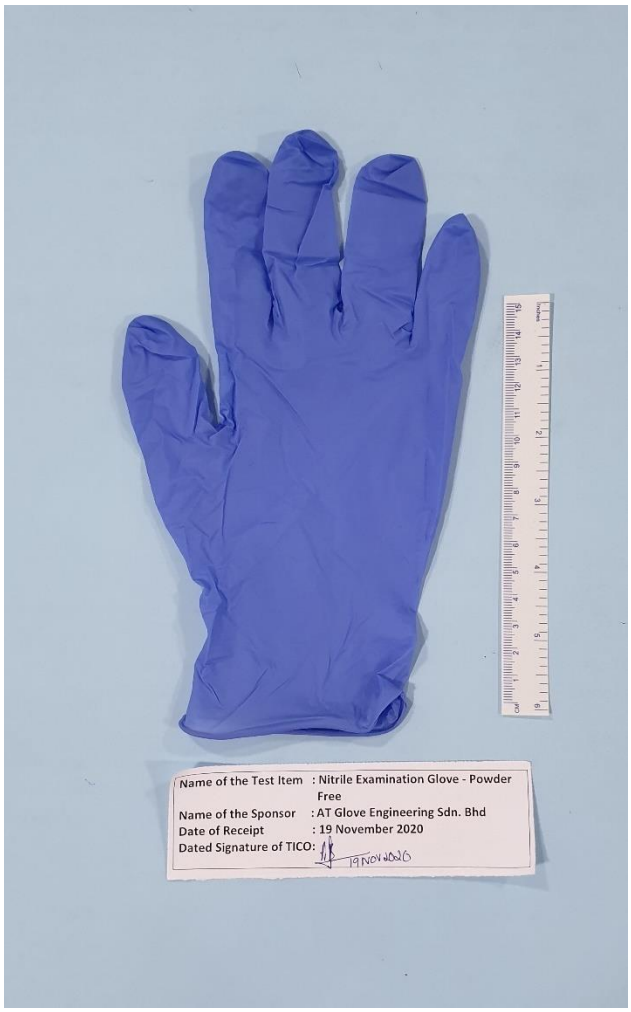
Total score = Sum of all the scores at test site (or) negative control site;
Primary Irritation (PI) Score = Total score divided by 6;
Source: ISO 10993-10:2010(E) Clause 6.3.6

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

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

Primary irritation index (PII) = Sum of all primary irritation scores divided by total number of animals
PII difference = PII of test site - PII of negative control site
Source: ISO 10993-10:2010(E) Clause 6.3.6

PHOTOGRAPH OF THE TEST ITEM



APPENDIX 1

CONCISE POSITIVE CONTROL STUDY DATA

Study number	000/043
Study title	Skin Irritation Study in New Zealand White Rabbits
Study start date	05 August 2020
Experiment start date	06 August 2020
Experiment completion date	22 August 2020
Study completion date	31 August 2020

INTRODUCTION

Skin irritation is a key toxicity endpoint to assess biocompatibility of medical devices. An assessment is made for testing the potential of the material under test to produce dermal irritation in rabbits following topical application. This study is a positive control trial, which is conducted once in every three months in GLR Laboratories Private Limited, to validate our routine procedures.

OBJECTIVE

This skin irritation study was conducted to demonstrate the positive response of Sodium Lauryl Sulphate in New Zealand White Rabbits.

DETAILS OF POSITIVE CONTROL ITEM [Sodium Lauryl Sulphate]

Appearance/Colour	Form: Rods, Colour: White
Manufacturer	Sigma Aldrich
Batch No.	0000009635
Manufacture Date	Not available
Expiry Date	August 2022
Concentration used in study	20% w/v Sodium Lauryl Sulphate

METHODOLOGY

This study was performed based on ISO 10993-10:2010(E) and OECD 404 standard.

Two grams of Sodium Lauryl Sulphate was dissolved in 9 mL of 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 15 h and 15 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

Table 1: 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	0	1	0	0	0	1	0	1	0	0	0	1	0	1	0	0	0
	24	1	1	2	0	0	0	1	2	3	0	0	0	1	1	2	0	0	0
	48	1	2	3	0	0	0	2	2	4	0	0	0	2	2	4	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	1	2	0	0	0	1	0	1	0	0	0	0	1	1	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	1	2	0	0	0	1	2	3	0	0	0	1	1	2	0	0	0
	48	1	1	2	0	0	0	2	2	4	0	0	0	2	1	3	0	0	0
	72	1	2	3	0	0	0	2	1	3	0	0	0	1	1	2	0	0	0
	Day 7	0	0	0	0	0	0	1	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 10993-10:2010(E) Clause 6.3.6

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	2	16	2.7	3	21	3.5	2	17	2.8
		48	3			4			4		
		72	4			4			4		
	Oedema formation	24	2			3			2		
		48	2			4			3		
		72	3			3			2		
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) Clause 6.3.6

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	3.0
Test item site	2.7	3.5	2.8	3.0	

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) Clause 6.3.6

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 3.0 and hence concluded as a moderate irritant under the conditions of the present study.

Summary of Positive Control Trial (GLR Study number 000/043)

Study number	Study start date	Experiment start date	Experiment completion date	Study completion date	Agent used	Result
000/043	05 August 2020	06 August 2020	22 August 2020	31 August 2020	20% Sodium Lauryl Sulphate	Moderate irritant

The next positive control trial has been initiated in November 2020.

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 10993-10:2010(E)

RESPONSIBLE PERSONNEL

Mr. K. Sakthivel, MSc (Biotech)	Study Director
Ms. D. Nandhini, BPharm	Study Scientist
Ms. S. Bharkavi, MSc	Study Scientist
Dr. D. Yogaraj, MVSc	Study Scientist
Dr. S. Kavirajan, MVSc	Veterinarian
Mr. M. Karthick, MSc	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 and
- 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).