AMENDED FINAL REPORT

Skin Irritation Test in New Zealand White Rabbits of TAGLUS ALIGNER AND RETAINER (Batch No.: 91021071-1) as per ISO 10993-10:2010 (E).

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AMENDED FINAL REPORT

PRODUCT NAME:

TAGLUS ALIGNER AND RETAINER (Batch No.: 91021071-1)

STUDY TITLE

Skin Irritation Test in New Zealand White Rabbits

PROJECT NUMBER
4788877994

TEST FACILITY
GLR Laboratories Private Limited
444 Gokulam Street
Mathur, Chennai - 600 068
Tamil Nadu, India

STUDY NUMBER
073/266

Report Issued Date:
02 May 2019

Amended Report Issued Date:
30 May 2019

STUDY SPONSOR

Vedia Solutions, Div of Laxmi Dental Export Pvt Ltd.,
103, Akruti Arcade, J P Road, Opp A H Wadia School
Andheri West, Mumbai 400053, Maharashtra, India

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STUDY DIRECTOR AUTHENTICATION STATEMENT

TAGLUS ALIGNER AND RETAINER (Batch No.: 91021071-1):
Skin Irritation Test in New Zealand White Rabbits

This study was performed in accordance with the agreed study plan and using GLR Laboratories Private Limited's standard operating procedures unless otherwise stated, and the study objective was achieved. I accept responsibility for the work and generated data, that are scientifically acceptable and valid, and this report provides a true and accurate record of the results obtained.

This study was performed in compliance with the OECD Principles of Good Laboratory Practice* ENV/MC/CHEM (98)17 (Revised 1997, issued January 1998).

Mr. S. Balaji, MSc Study Director GLR Laboratories Private Limited	30 May 2019 Study Completion Date

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

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QUALITY ASSURANCE STATEMENT

This study report has been reviewed by the Quality Assurance Unit of GLR Laboratories Private Limited, based on OECD Principles of GLP, Study Plan, Raw Data, and applicable Standard Operating Procedures.

This statement confirms that the study report accurately reflects raw data.

The summary of inspections performed during the study are as follows:

S. No.	Type of Inspection	Date of Inspection	Phase(s) of Study Inspected	Date of Reporting to Management, Study Director (Inspection Report No.)
1	Study Based Inspection	23 February 2019	Draft Study Plan	23 February 2019 (SBI/073/266/001)
2	Study Based Inspection	12 March 2019	Definitive Study Plan	12 March 2019 (SBI/073/266/002)
3	Study Based Inspection	03 April 2019	Test Item Extracts Application	03 April 2019 (SBI/073/266/003)
4	Study Based Inspection	06 April 2019	Grading of Skin Reaction	06 April 2019 (SBI/073/266/004)
5	Study Based Inspection	29 April 2019	Draft Report	29 April 2019 (SBI/073/266/005)
6	Study Based Inspection	02 May 2019	Final Report	02 May 2019 (SBI/073/266/006)
7	Study Based Inspection	30 May 2019	Amended Final Report	30 May 2019 (SBI/073/266/007)

G. Velmani

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

30 May 2019

Date

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TEST FACILITY MANAGEMENT STATEMENT

TAGLUS ALIGNER AND RETAINER (Batch No.: 91021071-1):
Skin Irritation Test in New Zealand White Rabbits

This is to certify that, the GLR test facility management appointed the Study Director for this study and provided him with all necessary facilities and resources for the proper conduct of this study, both in terms of GLP and scientific integrity.

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

30 May 2019
Date

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PEER REVIEW STATEMENT

This is to certify that I have reviewed the raw data and report along with the study director and agree with the scientific conclusions made.

T S Kumar

30 May 2019

Dr. T. S. Kumaravel, MD, PhD, DABT
American Board Certified and UK Registered Toxicologist
Chairman, GLR Laboratories Private Limited

Date

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REASON FOR AMENDMENT

As per sponsor request to update the study sponsor address in the final report page number 2 of 28.

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SUMMARY

Skin irritation potential of TAGLUS ALIGNER AND RETAINER (Batch No.: 91021071-1), supplied by Vedia Solutions, Unit of Laxmi Dental Exports Pvt Ltd, was evaluated in male New Zealand White rabbits.

TAGLUS ALIGNER AND RETAINER (Batch No.: 91021071-1) is intended for the prevention and correction of malpositioned teeth and jaws. The supplied test item (translucent flexible circular disc) is used to manufacture the taglus aligner and retainer. The dimensions are: diameter - 12.5 cm and thickness - 0.0762 / 0.102 cm (as stated by sponsor). It is a surface device which comes in contact with skin. The duration of contact is less than 24 hours.

Test item was freshly extracted in the ratio of 3 cm^2 / mL (since the thickness of the test item is more than 0.5 mm) using physiological saline (polar solvent) and sesame oil (non-polar solvent) at 37 ºC for 72 h under sterile conditions. The total surface area (both sides) of the one test item was approximately 245 cm^2 (as calculated in our laboratory). Polar extract was freshly prepared by extracting 245 cm^2 of test item in 81.7 mL of physiological saline. Similarly, non-polar extract was freshly prepared by extracting 245 cm^2 of test item in 81.7 mL of sesame oil, under sterile conditions. Solvent controls were also subjected to the similar extraction conditions in separate sterile containers. This fulfils the requirement of ISO 10993-12:2012 (E).

At the end of extraction, the extracts and solvent controls were clear, there was no change in the colour and no particulates were found (pre-and post-extraction). Hence, 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 hour 29 minutes of preparation and were considered stable during this time.

Twenty-one hours and fifty-four minutes prior to the commencement of experiment, all the six male rabbits were gently clipped free of their fur on the dorsal side, for an area of 10 cm x 15 cm on both sides of the spinal cord. The test item extract (0.5 mL) was applied to the sterile absorbent gauze measuring approximately 6.25 cm^2 (2.5 cm x 2.5 cm) and placed topically at the test sites of the fur clipped area of rabbit skin, in the dorsal region on the left cranial end and right caudal end of six rabbits (Animal no. 1, 2 and 3 were tested with physiological saline extract and Animal no. 4, 5 and 6 were tested with sesame oil extract).

Similarly, 0.5 mL of physiological saline / sesame oil was applied to the sterile absorbent gauze measuring 6.25 cm^2 (2.5 cm x 2.5 cm) and placed topically in the dorsal

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region on the right cranial end and left caudal end (control sites) 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 semi-occlusive dressing with means of non-irritant adhesive tape for 4 h. The test item and control item patches were removed at the end of 4 h exposure and the treatment sites were marked with non-irritant permanent marker ink.

All the animals were observed for three consecutive days for morbidity, mortality, skin reactions and abnormal clinical signs and symptoms following the patch removal.

The susceptibility of New Zealand White rabbits to a known irritant, 10% Sodium Lauryl Sulphate has been established at GLR Laboratories Private Limited and confirmed once in every three months. The last such positive control trial study was completed on 25 April 2019 and gave a "moderate irritant" reaction. The next positive control trial will be conducted in July 2019. Positive control trial conducted within the test facility indicated a clear positive result and the solvent control used in the study gave a mean irritation score of '0'. Therefore, the assay was considered valid.

No mortality or morbidity were observed in the experimental animals. All the animals showed increase in body weight 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'.

Based upon the results obtained in this study and in line with ISO 10993-10:2010 (E) it is concluded that, the given test item TAGLUS ALIGNER AND RETAINER (Batch No./Lot No.: 91021071-1), supplied by Vedia Solutions, Unit of Laxmi Dental Exports Pvt Ltd, is a non-irritant.

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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.

The test selection and methods used in this study were based on the following standards:

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 17025:2005(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. June 16, 2016.

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OBJECTIVE

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

STUDY DATES

Study Start Date	12 March 2019
Experiment Start Date	29 March 2019
Experiment Completion Date	06 April 2019

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

TEST ITEM DETAILS

The test item, TAGLUS ALIGNER AND RETAINER (Batch No.: 91021071-1) was received at GLR Laboratories Private Limited on 14 February 2019 and stored at room temperature (20.1 to 24.7 °C) until use. The following test item information provided by the sponsor were considered adequate.

Test Item	TAGLUS ALIGNER AND RETAINER
Batch/Lot No.	91021071-1
Manufacture Date	17 January 2019
Expiry Date	15 January 2021
Appearance	Translucent flexible circular disc
Ingredients	PET G
Temperature Stability	37 °C
Sterility	Non-Sterile

CONTROL ITEM DETAILS

Positive Control	10% w/v Sodium Lauryl Sulphate	
	Manufacturer	Avantor Performance Material India Ltd
	Batch No.	J159K18
	Manufacture date	October 2018
	Expiry date	November 2023

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	No animals were used for positive control in this study. Positive control trials for irritation are conducted once in every three months in GLR laboratories Private Limited. This strain of rabbits gives a clear positive response to 10% Sodium Lauryl Sulphate (SLS) in water. The details of positive control trials are provided in Appendix 1.
Solvent Controls	Physiological saline
	Manufacturer: Eurolife Healthcare Pvt. Ltd. Batch No.: 10170838B Expiry Date: September 2020 Appearance: Colourless clear solution
	Sesame oil
	Manufacturer: Sigma-Aldrich Lot No.: MKCG9353 Expiry Date: February 2024 Appearance: Yellow coloured viscous liquid

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

Description of the test item

TAGLUS ALIGNER AND RETAINER (Batch No.: 91021071-1) is intended for the prevention and correction of malpositioned teeth and jaws. The supplied test item (translucent flexible circular disc) is used to manufacture the taglus aligner and retainer. The dimensions are: diameter - 12.5 cm and thickness - 0.0762 / 0.102 cm (as stated by sponsor). It is a surface device which comes in contact with skin. The duration of contact is less than 24 hours.

TEST SYSTEM

Species	*Oryctolagus cuniculus* (Rabbit)
Strain	New Zealand White
Weight (g) (at the time of dosing)	2180.4 to 2493.3
Sex	Male

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Source	Sainath Agencies, 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	5 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)	19.9 to 22.2
Relative humidity (%)	43 to 62
Housing	Animals were housed individually in stainless steel rabbit cages.
Method of identification	Animals were identified using cage cards indicating cage no., study no., species, strain, animal no., sex, age/body weight, dose, signature and individual ear marking.
Feed	Commercial rabbit pellet feed (Amrut feeds)
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. Absorbent paper paddings, used to collect excreta and urine, were changed daily.
Photoperiod	12: 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.

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Personnel	Appropriately qualified and trained associates 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 was freshly extracted in the ratio of 3 cm^2 / mL (since the thickness of the test item is more than 0.5 mm) using physiological saline (polar solvent) and sesame oil (non-polar solvent) at 37 ºC for 72 h under sterile conditions. The total surface area (both sides) of the one test item was approximately 245 cm^2 (as calculated in our laboratory). Polar extract was freshly prepared by extracting 245 cm^2 of test item in 81.7 mL of physiological saline. Similarly, non-polar extract was freshly prepared by extracting 245 cm^2 of test item in 81.7 mL of sesame oil, under sterile conditions. Solvent controls were also subjected to the similar extraction conditions in separate sterile containers. This fulfils the requirement of ISO 10993-12:2012 (E).

At the end of extraction, the extracts and solvent controls were clear, there was no change in the colour and no particulates were found (pre-and post-extraction). Hence, 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 hour 29 minutes of preparation and were considered stable during this time.

The details of extract preparation are given below,

Extract	Extraction vehicle	Surface area (cm^2) of the test item taken	Volume of vehicle (mL)	Extract preparation start time	Extract preparation end time	Appearance of extracts*
Polar Extract	Physiological saline	245#	81.7	09.10 a.m. on 31 Mar 2019	09.21 a.m. on 03 Apr 2019	Clear colourless solution, no particulates
Polar Solvent Control	Physiological saline	NA	10			Clear colourless solution, no particulates
Non-polar Extract	Sesame oil	245#	81.7			Yellow coloured viscous liquid, no particulates
Non-polar Solvent Control	Sesame oil	NA	10			Yellow coloured viscous liquid, no particulates

*Extraction vehicles did not undergo any colour changes during the extraction process; NA-Not applicable;
Total surface area (both sides) of the one test item was approximately 245 cm^2 (as calculated in our laboratory).

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The pH of the polar extract was 7.03. Therefore, the extract was found suitable to conduct skin irritation test in rabbits. The pH of the oil extract cannot be measured, but it is assumed acceptable.

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 application to determine skin irritation in evaluating the biocompatibility of medical devices.

The animals with healthy intact skin were selected in this study. Twenty-one hours and fifty-four minutes prior to the commencement of the experiment, fur from all the three rabbits were gently clipped free on their backs, for an area of 10 cm x 15 cm on both sides of the spinal cord.

Test procedure

All the six rabbits (3 animals each was used for physiological saline and sesame oil extracts) was clipped free of fur on dorsal side from an area of, approximately 10 cm x 15 cm on either side of the vertebral column about, twenty-one hours fifty-four minutes prior to commencement of the study.

Topical Application

The test item extract (0.5 mL) was applied to the sterile absorbent gauze (Make: Liv Medica products Pvt Ltd; B. No: S0360617; Expiry Date: May 2022), measuring approximately 6.25 cm^2 (2.5 cm x 2.5 cm) and placed topically at the test sites of the fur clipped area of rabbit skin, in the dorsal region on the left cranial end and right caudal end of six rabbits (Animal no. 1, 2 and 3 were tested with physiological saline extract and Animal no. 4, 5 and 6 were tested with sesame oil extract). Similarly, 0.5 mL of physiological saline / sesame oil was applied to the sterile absorbent gauze measuring 6.25 cm^2 (2.5 cm x 2.5 cm) 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 as shown in the following figure.

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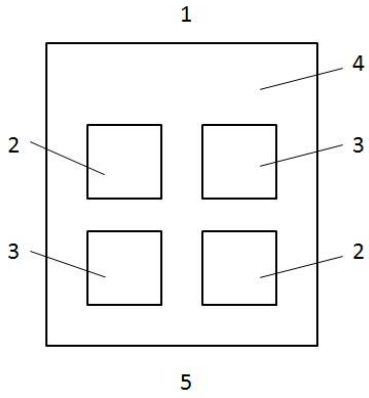

1. Cranial end; 2. Test site; 3. Control site; 4. Clipped dorsal region; 5. Caudal end.
Source: ISO 10993: Part 10: 2010 (E)

The application sites were covered with an absorbent gauze patch 2.5 cm x 2.5 cm and the patches were loosely held in contact with the skin by semi-occlusive dressing with means of non-irritant adhesive tape [Make: 3M India Limited; Batch No.: R06160302; Expiry Date: June 2021] for a duration of 4 h. 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.

OBSERVATIONS

Mortality & Morbidity

All the 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 at the time of dosing and experiment completion date.

Clinical observations

All 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

All animals were macroscopically examined for signs of erythema and oedema, visually with naked eyes. Skin reactions were graded and recorded at 1 h, and then at 24 h, 48 h and 72 h following the patch removal according to ISO 10993-10:2010 (E).

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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: Part 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.

Euthanasia & Necropsy

Animals were euthanized by Ketamine and Xylazine injection at the end of the experiment.

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 24, 48 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 of the test item and control was obtained by adding the scores of the individual animals and divided by the total number of three 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).

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ACCEPTANCE CRITERIA

The assay is to be considered valid if all the following criterions are met:

1. Positive control trial conducted within the test facility should indicate a clear positive result.
2. Solvent control used in the study should give a mean irritation score of 0 to 0.4.

RESULTS

Positive control trial conducted within the test facility indicated a clear positive result and the solvent control used in the study gave a mean irritation score of 0. Therefore, the assay was considered valid.

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

Body Weight
All the animals showed an increase in body weight at the end of the experiment. 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, 24, 48 and 72 h following patch removal are given in Table 2 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.

Euthanasia and Necropsy
Animals were euthanized by Ketamine and Xylazine injection at the end of the experiment. No gross pathology was carried out since no animals were found dead or in moribund condition.

CONCLUSION

Based upon the results obtained in this study and in line ISO 10993-10:2010 (E) it is concluded that, the given test item TAGLUS ALIGNER AND RETAINER (Batch No.: 91021071-1), supplied by Vedia Solutions, Unit of Laxmi Dental Exports Pvt Ltd is a non-irritant.

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REFERENCES

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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 17025:2005(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. June 16, 2016.

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Table 1: Individual body weights

Animal number	Sex	Individual body weights (grams)		Increase in body weight (grams)
		At the time of dosing	At the end of experiment	
1	M	2342.4	2368.0	25.6
2		2431.6	2460.1	28.5
3		2180.4	2212.5	32.1
4		2493.3	2525.4	32.1
5		2274.5	2306.3	31.8
6		2350.9	2374.2	23.3

M-Male

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

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Table 3: 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 of test item = Test - Control; PI Score = Total score divided by 6;
Source: ISO 10993-10:2010 (E) Clause 6.3.6

Table 4: Calculation for Primary Irritation Index and Primary Irritation difference by using Primary Irritation Score

Animal number	1	2	3	PII* (A)	PII difference#
Control site	0	0	0	0	0
Test item site	0	0	0	0	

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PHOTOGRAPH OF THE TEST ITEM

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APPENDIX 1

CONCISE POSITIVE CONTROL STUDY DATA

Study number	000/031
Study title	Skin Irritation Study in New Zealand White Rabbits
Study start date	30 March 2019
Experiment start date	06 April 2019
Experiment completion date	22 April 2019
Study completion date	25 April 2019

INTRODUCTION

Skin irritation is a key toxicity endpoint to assess the 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, conducted once in every three months in GLR Laboratories Private Limited, to validate the above procedure.

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	White powder
Manufacturer	Avantor Performance Material India Ltd
Batch No.	J159K18
Manufacture Date	October 2018
Expiry Date	November 2023.
Concentration used in study	10% w/v Sodium Lauryl Sulphate

METHODOLOGY

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

One gram of Sodium Lauryl Sulphate was dissolved in 9 ml of distilled water and made up to 10 ml to obtain 10% w/v Sodium Lauryl Sulphate solution. Three rabbits were clipped free of fur on dorsal side from an area of approximately 10 cm x 15 cm on both

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sides of the spinal cord approximately 16 h 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: Liv Medica Products Pvt Ltd; Batch No.: S0360617; Expiry date: May 2022) which was loosely held in contact with the skin by means of a suitable semi-occlusive dressing and non-irritant adhesive tape (3M India Limited; Batch No.: R06160302; Expiry Date: June 2021) 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	1	2	0	0	0	1	1	2	0	0	0	1	1	2	0	0	0
	24	1	1	2	0	0	0	2	1	3	0	0	0	2	2	4	0	0	0
	48	2	2	4	0	0	0	2	2	4	0	0	0	2	1	3	0	0	0
	72	2	2	4	0	0	0	1	2	3	0	0	0	1	1	2	0	0	0
	Day 7	1	1	2	0	0	0	1	1	2	0	0	0	1	0	1	0	0	0
Oedema formation	1	0	0	0	0	0	0	1	1	2	0	0	0	0	0	0	0	0	0
	24	0	1	1	0	0	0	1	0	1	0	0	0	2	1	3	0	0	0
	48	1	1	2	0	0	0	1	1	2	0	0	0	1	1	2	0	0	0
	72	1	0	1	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0
	Day 7	1	0	1	0	0	0	0	0	0	0	0	0	1	0	1	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

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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	14*	2.3	3	13*	2.1	4	15*	2.5
		48	4			4			3		
		72	4			3			2		
	Oedema formation	24	1			1			3		
		48	2			2			2		
		72	1			0			1		
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 of test item = Test - Control; 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	2.3
Test item site	2.3	2.1	2.5	2.3	

* Primary irritation index (sum of all primary irritation scores/number of animals)
# Primary irritation difference (Test of PII - Control of PII)
Source: ISO 10993-10:2010 (E) Clause 6.3.6

DISCUSSION

Based on the primary irritation index obtained, 10% 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 10% Sodium Lauryl Sulphate will also induce irritation in mucosal membranes. No separate animal experiments were performed in view of 3R's principles of animal testing.

CONCLUSION

Based on the results obtained, 10% w/v Sodium Lauryl Sulphate induced a primary irritation index of 2.3 compared to negative control. Hence it is concluded that the given test item Sodium Lauryl Sulphate is a moderate irritant under the conditions of the present study.

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Summary of Positive Control Trial (GLR Study number 000/031)

Study number	Study start date	Experiment start date	Experiment completion date	Study completion date	Agent used	Result
000/031	30 March 2019	06 April 2019	22 April 2019	25 April 2019	10% Sodium Lauryl Sulphate	Moderate irritant

The next positive control trial will be conducted in July 2019.

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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 Part 10: 2010(E)

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RESPONSIBLE PERSONNEL

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Ms. N. Narmadha, MSc, MPhil	Study Scientist
Mr. R.V. Venkataramanan, M Pharm	Study Scientist
Dr. D. Yogaraj, MVSc	Veterinarian
Mr. K. Sakthivel, MSc	Animal House In-charge

STUDY PLAN AMENDMENT

No amendments from the study plan were found during the conduct of the study.

STUDY PLAN DEVIATION

No deviations from the study plan were found during the conduct of the study.

ARCHIVE STATEMENT

All primary data, or authenticated copies thereof, slides (if applicable), tissue specimens (if applicable), a sample test items, 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 will be prepared and distributed as mentioned below:

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

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