FINAL REPORT

Skin Sensitization Test in Guinea Pigs (Guinea Pig Maximization Test) of TAGLUS ALIGNER AND RETAINER (Batch No.: 91021071-1) as per ISO 10993-10:2010 (E).

Kalyani Platina, 3rd Floor, Block I, EPIP Zone, Phase II, Whitefield,
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FINAL REPORT

PRODUCT NAME:

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

STUDY TITLE

Skin Sensitization Test in Guinea Pigs
(Guinea Pig Maximization Test)

PROJECT NUMBER
4788877994

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

STUDY NUMBER
073/267

REPORT ISSUED DATE:
29 May 2019

STUDY SPONSOR

Vedia Solutions, Unit of Laxmi Dental Exports Pvt Ltd
103 Akruti Arcade, JP Road,
Opposite 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 Sensitization Test in Guinea Pigs (Guinea Pig Maximization Test)

This study was performed in accordance with the agreed study plan, One definitive study plan amendment, with 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).

29 may 2019

Mr. R.V. Venkataramanan, M Pharm
Study Director
GLR Laboratories Private Limited

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 the OECD Principles of GLP, study plan, One definitive study plan amendment, 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 course of 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/073/267/001)
2	Study Based Inspection	11 March 2019	Definitive Study Plan	11 March 2019 (SBI/073/267/002)
3	Study Based Inspection	05 April 2019	Test Item Extract Administration Intradermal Phase	05 April 2019 (SBI/073/267/003)
4	Study Based Inspection	12 April 2019	Test Item Extract Application Topical Phase	12 April 2019 (SBI/073/267/004)
5	Study Based Inspection	26 April 2019	Test Item Extract Application Challenge Phase	26 April 2019 (SBI/073/267/005)
6	Study Based Inspection	29 April 2019	Scoring of Skin Reaction	29 April 2019 (SBI/073/267/006)
7	Study Based Inspection	28 May 2019	Definitive Study Plan Amendment	28 May 2019 (SBI/073/267/007)
8	Study Based Inspection	29 May 2019	Final Report	29 May 2019 (SBI/073/267/008)

G. Velmani

29 May 2019

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

Date

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TEST FACILITY MANAGEMENT STATEMENT

TAGLUS ALIGNER AND RETAINER (Batch No.: 91021071-1): Skin Sensitization Test in Guinea Pigs (Guinea Pig Maximization Test)

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.

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

29 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 Kumaravel

29 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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SUMMARY

Skin sensitization 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 guinea pigs using guinea pig maximization test (GPMT).

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 of the test item 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.

The test item extract was prepared at the ratio of 3 cm^2/mL (as thickness of the test item is more than 0.5 mm) of solvent at 37 ºC for 72 h under sterile condition. Total surface of test item is 245 cm^2 (as calculated in our laboratory). One test item was used for each extraction. For each intradermal induction, topical application and challenge phase, 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 condition. Solvent controls were also subjected to the similar extraction conditions. This fulfils the requirement of ISO 10993 part 12: 2012 (E).

At the end of extraction, the extracts and solvent controls were clear, no colour change 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 6 h and 15 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: 2010(E).

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No positive controls were included in this study. Positive control trials for sensitization are carried out once in every three months at GLR laboratories Private Limited in guinea pigs using 2,4-Dinitrochlorobenzene. The last such positive control trial for skin sensitization (GPMT) was completed on 26 March 2019 and gave a clear sensitizing reaction in 90% of treated animals (Appendix 3). The next positive control trial will be conducted in June 2019. No response was observed in solvent controls treated animals. Therefore, the assay was considered valid.

No mortality or morbidity were observed in any of the animals used in this study. All the animals showed an increase in body weight at the end of the experiment. No skin sensitization reactions were observed in both control and test sites. Therefore, no gross and histopathological examinations were performed.

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

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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 of medical devices.

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

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 sensitization potential of the test item extracts using guinea pig maximization test (GPMT).

STUDY DATES

Study Start Date	11 March 2019
Experiment Start Date	29 March 2019
Experiment Completion Date	29 April 2019

The study completion date is the date the final report is signed by the study director.

This study was performed inline with agreed study plan and one definitive study plan amendment.

TEST AND CONTROL 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 to 30 °C) until used.

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 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	
Sterility	Non - Sterile	
Temperature Stability	37^{o}C	
Positive Control	2,4-Dinitrochlorobenzene	
	Manufacturer	Sigma-Aldrich
	Batch No.	BCBS4201V
	Expiry Date	February 2023

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	No positive controls were included in this study. Positive control trials for sensitization are carried out once in every three months at GLR laboratories Private Limited in guinea pigs using 2,4-Dinitrochlorobenzene. The last such positive control trial for skin sensitization (GPMT) was completed on 26 March 2019 and gave a clear sensitizing reaction in 90% of treated animals (Appendix 3). The next positive control trial will be conducted in June 2019.
Solvent Control	Physiological saline and sesame oil

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

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	*Cavia porcellus* (Guinea pig)
Strain	Dunkin - Hartley
Weight range (g) (at the time of dosing)	318.51 to 476.08
Sex	Male
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	30
Number of groups	4
Number of animals per group	Physiological saline control: 5 Physiological saline extract: 10

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	Sesame oil control: 5 Sesame oil extract: 10
Acclimatization period	7 days
Justification for animal use	Guinea pigs were selected because there is a large volume of background data on this species. Recommended in ISO 10993-10: 2010 (E) standard guideline as an appropriate species to evaluate the 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.	08
Test room temperature (°C)	19.5 to 21.9
Relative humidity (%)	39 to 61
Housing	Animals were housed individually in standard clean polypropylene cages.
Method of identification	Animals were identified using cage cards indicating cage no., study no., species, strain, animal no., sex, age/body weight, group no., dose and signature.
Feed	Commercial Guinea pig pellet feed (VRK Nutritional Pellet Feeds)
Water	Purified drinking water supplemented with vitamin C was provided *ad libitum*.
Bedding material	Sterilized paddy husk
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. Certificate of contaminant analysis is attached.
Personnel	Appropriately qualified and trained associates were involved in this study.
Selection of animals	Only healthy young adults, previously unused animals were selected for this study.

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TEST METHOD

Preparation of the test item extracts

The test item extract was prepared at the ratio of 3 cm^2/mL (as thickness of the test item is more than 0.5 mm) of solvent at 37 ºC for 72 h under sterile condition. One test item was used for each extraction. For each intradermal induction, topical application and challenge phase, 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 condition. Solvent controls were also subjected to the similar extraction conditions. This fulfils the requirement of ISO 10993 part 12: 2012 (E).

The details of the solvents used are as follows:

Physiological saline (0.9% w/v Sodium Chloride solution)

Manufacturer	Eurolife Healthcare Private Limited
Batch No.	10170838B
Expiry date	September 2020
Appearance	Colourless clear solution

Sesame oil

Manufacturer	Sigma-Aldrich
Lot No.	MKCF9353
Expiry Date	February 2024
Appearance	Yellow coloured viscous liquid

Day 0: Intradermal induction phase

The required volume of extract was prepared freshly prior to dosing as given below.

Extract/Solvent	Extraction vehicle	Surface area (cm^2) of the test item taken	Volume of vehicle (mL)	Extract preparation start time	Extract preparation end time	Condition of extracts*
Polar solvent control	Physiological saline	NA	10	11.15 a.m. 02 Apr 2019	11.20 a.m. 05 Apr 2019	Colourless clear solution; no particulates
Polar extract	Physiological saline	245	81.7			Colourless clear solution; no particulates
Non-polar solvent control	Sesame oil	NA	10			Yellow coloured viscous liquid; no particulates
Non-polar extract	Sesame oil	245	81.7			Yellow coloured viscous liquid; no particulates

*extraction vehicles did not undergo any colour changes during the extraction process; NA-Not applicable.
The total surface area of the one test item is 245 cm^2 (as calculated in our laboratory)

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

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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 5 h and 20 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 given below.

Extract/Solvent	Extraction vehicle	Surface area (cm^2) of the test item taken	Volume of vehicle (mL)	Extract preparation start time	Extract preparation end time	Condition of extracts*
Polar solvent control	Physiological saline	NA	10	11.15 a.m. 09 Apr 2019	11.15 a.m. 12 Apr 2019	Colourless clear solution; no particulates
Polar extract	Physiological saline	245	81.7			Colourless clear solution; no particulates
Non-polar solvent control	Sesame oil	NA	10			Yellow coloured viscous liquid; no particulates
Non-polar extract	Sesame oil	245	81.7			Yellow coloured viscous liquid; no particulates

*extraction vehicles did not undergo any colour changes during the extraction process; NA-Not applicable.
The total surface area of the one test item is 245 cm^2 (as calculated in our laboratory).

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 5 h and 10 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 given below.

Extract/Solvent	Extraction vehicle	Surface area (cm^2) of the test item taken	Volume of vehicle (mL)	Extract preparation start time	Extract preparation end time	Condition of extracts*
Polar solvent control	Physiological saline	NA	10	10.55 a.m. 23 Apr 2019	11.00 a.m. 26 Apr 2019	Colourless clear solution; no particulates
Polar extract	Physiological saline	245	81.7			Colourless clear solution; no particulates
Non-polar solvent control	Sesame oil	NA	10			Yellow coloured viscous liquid; no particulates
Non-polar extract	Sesame oil	245	81.7			Yellow coloured viscous liquid; no particulates

*extraction vehicles did not undergo any colour changes during the extraction process; NA-Not applicable.
The total surface area of the one test item is 245 cm^2 (as calculated in our laboratory).

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,

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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 6 h and 15 min of preparation and were considered stable during this time.

Test Procedure

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

Intradermal Induction phase

Intradermal injections (with 1 mL syringe, Make: Hindustan Syringes & Medical Devices Ltd, Batch No: 713015AG32, Expiry Date: February 2022) of the test item extracts, solvent and Freund's Complete Adjuvant (FCA) (Sigma-Aldrich; Lot No. SLBV0593; Expiry date: March 2023) 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 (FCA) + 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) FCA + solvent (solution A)
Site B: Polar extract of test item 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 in day 6, the test area was treated with 10% sodium lauryl sulphate (Sigma-Aldrich; Batch No.: 0000009635; Expiry date: August 2022) in petroleum jelly (Make: HiMedia Laboratories Pvt Ltd; Lot. No: 0000314448; Expiry date: Nov 2022) to create local irritation.

On day 7, absorbent gauze measuring 8 cm^2 (Liv Medica Products Pvt Ltd; Batch No.: S0360617; Expiry date: May 2022) loaded with 0.5 mL of test item extract and solvent, respectively was applied topically to respective groups of guinea pigs, on the same site

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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. Patch measuring 8 cm^2 (Liv Medica Products Pvt. Ltd; Batch No.: S0360617; Expiry date: May 2022) soaked with 0.5 mL of test item extract was applied on the left side and the patch with 0.5 mL of the solvent was applied 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.

Justification for method of administration	The method of administration is in line with the ISO 10993-10: 2010 (E) standard. For the induction phase, intradermal injections and the topical application were employed. The challenge phase was accomplished by topical applications.

OBSERVATIONS

Mortality & Morbidity

All the animals were observed daily for mortality and morbidity throughout the experiment.

Body weight

Body weight of each animal was recorded at the time of 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 challenging patch. The challenge application sites were assessed for erythema and edema using Magnusson and Kligman scale (Appendix 2).

Euthanasia and Necropsy

All the animals were euthanized by carbon dioxide (CO_2) exposure at the end of the experiment. Since no abnormality were observed in any animals, necropsy was not performed.

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DATA EVALUATION

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

Skin reactions elicited in terms of incidence and severity of reactions between the test item extracts treated and solvent control groups will be compared.

Magnusson and Kligman grades of 1 or greater in the test group generally indicate sensitization, provided grades of less than 1 are seen in control animals. If grades of 1 or greater are noted in control animals, then the reactions of test animals which exceed the most severe reaction in control animals are presumed to be due to sensitization. If the response is equivocal, re-challenge may be performed to confirm the results from the first challenge. A re-challenge will be carried out 1 week to 2 weeks after the first challenge (induction phase) on the naive side of the animals. The outcome of the results will be presented as the frequency of positive challenge results in test and control animals. If equivocal responses remain, the study will be re-conducted using a minimum of 20 test and 10 control animals.

ACCEPTANCE CRITERIA

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

1. Positive control trial conducted within the test facility should give clear positive results.

2. No response should be observed in solvent control treated animals.

RESULTS

Positive control trial conducted within the test facility gave clear positive results (Appendix 3). No response was observed in solvent control treated animals. Therefore, the assay was considered as valid.

Mortality & Morbidity

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

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Body weight

All the animals showed an increase in body weight at the end of the experiment. Body weight of all animals recorded at the time of dosing and at the end of the experiment (Table 1).

Grading of skin reactions

Grading of skin reactions performed at 24 h and 48 h after removing the challenging patch are given in Table 2. Erythema observed at intradermal injection site 1 and site 3 in all the animals was due to FCA injection and it does not interfere with study results. No sensitization reactions were observed in animals treated with the negative control. No evidence of sensitization was seen in any of the test item extracts 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 10993-10:2010 (E) it is concluded that, the extracts of the given test item TAGLUS ALIGNER AND RETAINER (Batch No.: 91021071-1) supplied by Vedia Solutions, Unit of Laxmi Dental Exports Pvt Ltd is non-sensitizer.

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REFERENCES

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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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PHOTOGRAPH OF THE TEST ITEM

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Table 1: Individual body weights of all animals

Group	Sex	Animal No.	Weight (in grams)		
			At time of dosing	At the end of experiment	Increase in weight
G1	M	01	348.54	386.36	37.82
		02	432.36	467.93	35.57
		03	354.99	392.57	37.58
		04	432.58	466.59	34.01
		05	318.62	354.73	36.11
		Mean ± SD	**377.42± 52.10**	**413.64 ± 51.01**	**36.22 ± 1.56**
G2	M	06	474.10	513.62	39.52
		07	386.44	420.89	34.45
		08	331.66	364.69	33.03
		09	428.67	462.19	33.52
		10	447.87	487.14	39.27
		11	448.43	484.37	35.94
		12	334.02	372.18	38.16
		13	331.33	362.20	30.87
		14	394.49	429.16	34.67
		15	351.85	390.98	39.13
		Mean ± SD	**392.89 ± 54.48**	**428.74 ± 55.82**	**35.86 ± 3.03**
G3	M	16	439.27	477.23	37.96
		17	346.40	385.34	38.94
		18	451.62	483.14	31.52
		19	473.51	506.69	33.18
		20	428.14	458.57	30.43
		Mean ± SD	**427.79 ± 48.51**	**462.19 ± 46.27**	**34.41 ± 3.83**
G4	M	21	319.18	350.78	31.60
		22	476.08	507.21	31.13
		23	408.90	443.85	34.95
		24	318.51	356.23	37.72
		25	357.00	388.09	31.09
		26	392.38	430.87	38.49
		27	446.89	483.68	36.79
		28	423.15	462.43	39.28
		29	408.32	444.32	36.00
		30	328.31	364.99	36.68
		Mean ± SD	**387.87 ± 55.20**	**423.25 ± 55.31**	**35.37 ± 3.08**

M-Male; SD- Standard Deviation

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Table 2: Grading results 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		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		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		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

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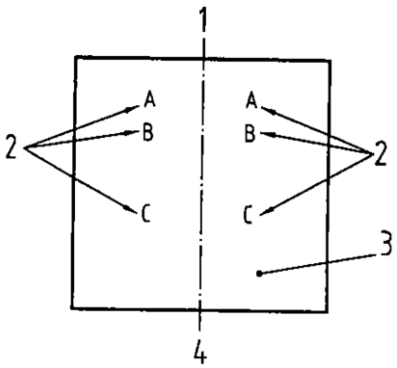
APPENDIX 1

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		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		Polar extract of Test item	Polar solvent & Polar extract of test item
G3	16-20		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		Non-po lar solvent	Non-polar solvent & Non-polar extract of test item
G4	21-30		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		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 21
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 10993-10:2010(E).

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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 10993-10:2010(E).

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APPENDIX 3

CONCISE POSITIVE CONTROL STUDY DATA

Study Number	000/030
Study Title	Skin sensitization test in guinea pigs (Guinea Pig Maximization Test)
Study Start Date	15 February 2019
Experiment Start Date	19 February 2019
Experiment Completion Date	21 March 2019
Study Completion Date	26 March 2019

OBJECTIVE

To demonstrate a positive response of 2,4-Dinitrochlorobenzene (Batch No. BCBS4201V) in guinea pigs using guinea pig maximization test at GLR Laboratories Private Limited. This data will serve as a positive control for all sensitization studies conducted at GLR Laboratories Private Limited for three months to validate our routine procedure.

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	February 2023

METHODOLOGY

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

Induction: Intradermal Injections

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

Similarly, 0.1 mL of intradermal injections were given to the fur clipped animals in the control group. Injection 1: a 1:1 mixture (v/v) of FCA & physiological saline (Solution

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A), injection 2: a 1:4 v/v acetone: sesame oil (Solution B) and injection 3: a 1:1 mixture of solution A and solution B.

Induction: Topical Application

Since DNCB was an irritant, 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 a patch (2 x 4 cm) fully-loaded with 0.2 mL of 0.25 % w/v of DNCB in a 1:4 v/v acetone: sesame oil and 0.2 mL of 1:4 v/v acetone: sesame 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. Patch soaked with 0.2 mL of 0.1 % w/v of DNCB in 1:4 v/v acetone: sesame oil was applied on left side and the patch soaked with 0.2 mL of 1:4 v/v acetone: sesame 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 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 hours after removing the patch, the challenge area was cleaned and closely-clipped. At 24 and 48 h after patch removal, the challenge application sites were scored using a Magnusson and Kligman 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	48 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	1	1
	9	0	0	0	0
	10	0	0	1	0
	11	0	0	1	1
	12	0	0	2	1
	13	0	0	1	1
	14	0	0	1	1
	15	0	0	1	0

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CONCLUSION

The results indicated that animals treated with 2,4-Dinitrochlorobenzene (Batch No. BCBS4201V), induced sensitization reactions in 90% of treated animals. Therefore, according to OECD Guidelines for Testing of Chemicals, 406 and ISO 10993, Part-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 (000/030)

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
15 February 2019	19 February 2019	21 March 2019	26 March 2019	0.025% w/v	0.25% w/v	0.1% w/v	1:4 v/v acetone: sesame oil	+ve in 9/10 animals	Grade 2 - Moderate and confluent erythema

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

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RESPONSIBLE PERSONNEL

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

STUDY PLAN AMENDMENT

One definitive study plan amendment was made to change the sponsor address as per sponsor request.

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 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).

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