eurofins | Medical Device Testing

Skin Sensitisation

(Guinea Pig Maximisation Test)

with a Nonpolar Extract of

TK-Soft mini Placeholder & TK-Soft mini Frictionelement

Study Plan

Version: Final

Eurofins Munich Study No.: 173383B

UL MDT Project # 11757924 3.1-1 & 11757924 3.1-2

Sponsor:

Si-tec GmbH Dentalspezialartikel
Leharweg 2
58313 Herdecke
Germany

Eurofins BioPharma Product Testing Munich GmbH
Behringstr. 6/8
D-82152 Planegg/Munich
Germany

Tel | +49 (0)89 899 650-0
Fax | +49 (0)89 899 650-11

in cooperation with

BSL BIOSERVICE Scientific Laboratories Munich GmbH
Behringstr. 6/8
D-82152 Planegg/Munich
Germany

Tel | +49 (0)89 899 974 9-0
Fax | +49 (0)89 899 974 9-25

1. Project Staff Signatures

Study Director
BSL BIOSERVICE
Scientific Laboratories Munich GmbH

Dr. Sandra Schmid

.....

Date: 12 Jun 2017

Management
BSL BIOSERVICE
Scientific Laboratories Munich GmbH

.....

Print Name: Dr. Angela Lutterbach

Date: 12 JUN 2017

Member of the
Quality Assurance Unit
Eurofins BioPharma
Product Testing Munich GmbH

.....

Print Name: Anna Boikina

Date: 13 JUN 2017

Sponsor's Representative

.....
THEO SCHLÜTER

Date: 26.07.2017

2. Contents

page

1. Project Staff Signatures 2

2. Contents 3

3. List of Tables 4

4. Preface 5

4.1. Abbreviations 5
4.2. General 6
4.3. Project Staff 6
4.4. Schedule 6

5. Quality Assurance 7

5.1. GLP Compliance 7
5.2. Guidelines 7
5.3. Amendment Procedures 8
5.4. Deviation Procedures 8
5.5. Archiving 8

6. Introduction 9

6.1. Justification for the Selection of the Test System 9
6.2. Justification for the Selection of the Test Method 9

7. Materials and Methods 10

7.1. Characterisation of the Test Item 10
7.2. Extraction of the Test Item 10
7.3. Control 11
7.4. Other Materials 11
7.5. Test System 11
7.5.1. Housing and Feeding Conditions 11
7.6. Preparation of the Animals 12
7.7. Induction: First Stage, Intradermal Injection 12
7.8. Induction: Second Stage, Topical Application 12
7.9. Challenge: Topical Application 13
7.10. Clinical Observation 13
7.11. Evaluation of Results 13

8. Reporting 14

9. Distribution of the Study Plan 15

10. References 16

10.1. Internal Eurofins Munich and BSL Munich SOPs 16
10.2. Literature and Guidelines 16

3. List of Tables

page

Table 1: Magnusson and Kligman Scale 13

4. Preface

4.1. Abbreviations

AAALAC	Association for Assessment and Accreditation of Laboratory Animal Care
BGBl.	Bundesgesetzblatt *(Federal Law Gazette)*
BSL Munich	BSL BIOSERVICE Scientific Laboratories Munich GmbH
DIN	Deutsches Institut für Normung *(German Institute for Standardisation)*
e.g.	exempli gratia *(for example)*
EN	Europäische Norm *(European standard)*
Eurofins Munich	Eurofins BioPharma Product Testing Munich GmbH
EWG	Europäische Wirtschaftsgemeinschaft *(European Economic Community, EEC)*
GLP	Good Laboratory Practice
GmbH	Gesellschaft mit beschränkter Haftung *(company with limited liability)*
IEC	International Electrotechnical Commission
ISO	International Organisation for Standardisation
No.	number
Nr.	Nummer *(number)*
OECD	Organisation for Economic Cooperation and Development
QAU	Quality Assurance Unit
SOP	Standard Operating Procedure
SPF	specific-pathogen free
v/v	volume per volume

4.2. General

Sponsor:	Si-tec GmbH Dentalspezialartikel Leharweg 2 58313 Herdecke Germany
Study Monitor:	Thilo Schlüter
Contractor:	UL International GmbH UL MDT Grenzenstr. 13 88416 Ochsenhausen Germany
Test Facility:	BSL BIOSERVICE Scientific Laboratories Munich GmbH Behringstr. 6/8 82152 Planegg Germany
Eurofins Munich Study No.:	173383B
Test Item:	TK-Soft mini Placeholder & TK-Soft mini Frictionelement
Title:	Skin Sensitisation (Guinea Pig Maximisation Test) with a Nonpolar Extract of TK-Soft mini Placeholder & TK-Soft mini Frictionelement

4.3. Project Staff

Study Director:	Dr. Sandra Schmid BSL BIOSERVICE Scientific Laboratories Munich GmbH Behringstr. 6/8 82152 Planegg Germany
Team Leader Operational QA GLP:	Uwe Hamann Eurofins BioPharma Product Testing Munich GmbH Behringstr. 6/8 82152 Planegg Germany

4.4. Schedule

Arrival of the Test Item:	12 May 2017
Study Initiation Date:	date of the study director's signature
Proposed Experimental Starting Date:	June/July 2017
Proposed Experimental Completion Date:	July/August 2017
Proposed Study Completion Date:	August 2017

5. Quality Assurance

5.1. GLP Compliance

This study will be conducted to comply with:

Chemikaliengesetz ("Chemicals Act") of the Federal Republic of Germany, Appendix 1 to § 19a as amended and promulgated on August 28, 2013 (BGBl. I S. 3498) [1].

Konsens-Dokument der Bund-Länder-Arbeitsgruppe Gute Laborpraxis ("Consensus Document of the National and Länder Working Party on Good Laboratory Practice") on the archiving and storage of records and materials, 5 May 1998 [2].

OECD Principles of Good Laboratory Practice (as revised in 1997); OECD Environmental Health and Safety Publications; Series on Principles of Good Laboratory Practice and Compliance Monitoring - Number 1. Environment Directorate, Organisation for Economic Co-operation and Development, Paris 1998 [3].

The OECD Principles of Good Laboratory Practice are accepted by regulatory authorities throughout the European Community, USA and Japan.

This study will be assessed for compliance with the study plan and the Standard Operating Procedures of Eurofins Munich and BSL Munich. The study and/or the test facility are periodically inspected by the Quality Assurance Unit according to the corresponding SOPs. These inspections and audits are carried out by the Quality Assurance Unit of Eurofins Munich which is independent of the staff involved in the study. A signed quality assurance statement listing all performed audits will be included in the report.

BSL Munich is accredited by the Deutsche Akkreditierungsstelle GmbH (DAkkS) according to guideline 90/385/EWG [4], 93/42/EWG [5] and DIN EN ISO/IEC 17025 [6]. The accreditation applies only to test methods listed in the accreditation scope [D-PL-20255-01-00]. The applied test method fulfills this requirement.

5.2. Guidelines

This study will follow the procedures indicated by internal Eurofins Munich and BSL Munich SOPs and the following internationally accepted guidelines and recommendations:

Biological evaluation of medical devices

ISO 10993-1: 2009 "Biological evaluation of medical devices – Part 1: Evaluation and testing within a risk management process" [7]

ISO 10993-2: 2006 "Biological evaluation of medical devices – Part 2: Animal welfare requirements" [8]

ISO 10993-10: 2010 "Biological evaluation of medical devices – Part 10: Tests for irritation and skin sensitization" [9]

ISO 10993-12: 2012 "Biological evaluation of medical devices – Part 12: Sample preparation and reference materials" [10]

Procedures and facilities comply with the requirements of Directive 2010/63/EU [11] and the national legislation defined in the animal protection law concerning the protection of animals used for experimental and other scientific procedures [12].

5.3. Amendment Procedures

This study plan can be amended at the discretion of the study director and/or in consultation with the study monitor. Changes or revisions of this study plan will be documented for stated reasons, signed and dated by the study director. All such amendments will be sent to the sponsor for approval and will be retained with the original study plan.

5.4. Deviation Procedures

Unplanned changes to the study plan and/or SOPs will be documented in the raw data. The chapter "Deviations from the Study Plan" in the report will reflect every deviation and its anticipated effect on the outcome of the study.

5.5. Archiving

For a period of 15 years (or shorter if in compliance with the GLP regulations) Eurofins Munich will store the records, materials and specimens in their scientific archives according to the GLP regulations.

The following records have to be stored according to the GLP regulations:

A copy of the final report, the study plan and a documentation of all raw data generated during the conduct of the study (documentation forms as well as any other notes of raw data, printouts of instruments and computers) and the correspondence with the sponsor concerning the study. Any document relating to the study will be discarded only with the prior consent of the sponsor.

The following materials and samples have to be stored according to the period of time specified in the GLP regulations:

A retained sample of the test item will be archived according to the GLP regulations, if possible, and will be discarded without the sponsor's prior consent.

Other materials and specimens have to be stored according to the GLP regulations and disposed of after the respective archiving period with the sponsor's prior consent.

Unless otherwise agreed in writing, the remaining test item will be discarded three months after the release of the report.

6. Introduction

6.1. Justification for the Selection of the Test System

The test for delayed-type hypersensitivity is performed on the guinea pig. It has been the animal of choice for predictive sensitisation tests for several decades. The assessment of the test item to produce skin sensitisation is achieved through multiple exposure under standardised conditions.

This test is appropriate because exposure to even minute amounts of potential leachables can result in allergic or sensitisation reactions.

6.2. Justification for the Selection of the Test Method

No validated *in vitro* method is available for assessing sensitisation potential.

7. Materials and Methods

7.1. Characterisation of the Test Item

The identity of the test item was inspected upon delivery at the test facility (e.g. test item name, batch no. and additional data were compared with the label) based on the following specifications provided by the sponsor.

Test item no. 1

Name:	TK-Soft mini Placeholder
Batch No.:	13795
Physical State:	solid
Composition:	Hostaform C9021, color service UN6037
Sterility:	non sterile
Storage Conditions:	room temperature
Calculated Surface as provided by the Sponsor:	0.29 cm^2
Expiry Date:	not applicable
Safety Precautions:	The routine hygienic procedures will be sufficient to assure personnel health and safety.

Test item no. 2

Name:	TK-Soft mini Frictionelement
Batch No.:	13794
Physical State:	solid
Composition:	Hostaform C9021, color service UN5063
Sterility:	non sterile
Storage Conditions:	room temperature
Calculated Surface as provided by the Sponsor:	0.44 cm^2
Expiry Date:	not applicable
Safety Precautions:	The routine hygienic procedures will be sufficient to assure personnel health and safety.

Additional specifications, concerning the test item, provided by the sponsor, can be part of the report and do not require an amendment to study plan.

7.2. Extraction of the Test Item

The extraction of the test item will be performed according to ISO 10993-12 [10].

On sponsor's request, the TK-Soft mini Placeholder and the TK-Soft mini Frictionelement will be pooled in equal shares for the extraction.

In total a ratio of 4 g of sample to 20 mL of extraction medium will be used.

Extraction conditions:	50 ± 2° C for 72 ± 2 h, under agitation
Extraction medium:	cottonseed oil, e.g. Sigma

7.3. Control

The extraction medium cottonseed oil will be incubated under agitation at 50° ± 2° C for 72 ± 2 h and will serve as negative control.

7.4. Other Materials

Freund's Complete Adjuvant

Gauze patches, EN 14079, Type 17, cut to 2.5 cm x 2.5 cm

Blenderm, hypoallergenic surgical tape

Elastomull haft, hypoallergenic elastic bandage

Na-lauryl sulphate

Vaseline

Depilatory cream, if necessary

7.5. Test System

Species/strain:	healthy guinea pigs, e.g. Crl: HA (full barrier)
Source:	e.g. Charles River, D-97633 Sulzfeld
Sex:	female (non-pregnant, nulliparous) or male
Body weight at the beginning of the study:	300 - 500 g, if technically possible
Number of animals:	10 animals test group, 5 animals negative control group

The animals will be derived from a controlled full-barrier maintained breeding system (SPF). According to the German Act on Animal Welfare [12] the animals are bred for experimental purposes.

Due to historical data and for animal welfare, additional animals for preliminary tests will only be used if considered necessary. This would be the case if the intradermal induction phase shows irritation.

This study will be performed in an AAALAC-accredited laboratory. According to German animal protection law, the study type has been reviewed and accepted by local authorities. Furthermore, the study has been subjected to Ethical Review Process and was authorised by the Bavarian animal welfare administration.

7.5.1. Housing and Feeding Conditions

- Semi barrier in an air-conditioned room
- Temperature: 22 ± 3 °C
- Relative humidity: 55 ± 10%
- Artificial light, sequence being 12 hours light, 12 hours dark
- Air change: at least 10 x / hour
- Free access to irradiated hay briquettes or autoclaved hay and to Altromin 3123 maintenance diet for guinea pigs, rich in crude fibre

- Free access to tap water (drinking water, municipal residue control, microbiological controls at regular intervals)
- The animals will be kept in groups in Terluran - cages on Altromin saw fibre bedding
- Certificates of food, water and bedding are filed for two years at BSL Munich and afterwards archived at Eurofins Munich
- Adequate acclimatisation period (at least 5 days)

7.6. Preparation of the Animals

The animals will be randomly selected and assigned to the treatment groups.

Identification will be ensured by individual marking.

7.7. Induction: First Stage, Intradermal Injection

Three pairs of intradermal injections of 0.1 mL volume will be given in the shoulder region which was cleared of hair so that one of each pair lies on each side of the midline.

Test Group:

Injection 1:	Freund's Complete Adjuvant, 1 + 1 diluted with cottonseed oil
Injection 2:	test item extract at a concentration of 100%
Injection 3:	test item extract at a concentration of 50% (v/v) in Freund's Complete Adjuvant, 1 + 1 diluted with cottonseed oil

Control Group:

Injection 1:	Freund's Complete Adjuvant, 1 + 1 diluted with cottonseed oil
Injection 2:	extraction medium at a concentration of 100%
Injection 3:	extraction medium at a concentration of 50% (v/v) in Freund's Complete Adjuvant, 1 + 1 diluted with cottonseed oil

The injections 1 and 2 will be given close to each other and nearest to the head, while injection 3 will be given toward the caudal part of the test area.

7.8. Induction: Second Stage, Topical Application

Test Group and Control Group

Approximately twenty-four hours before the topical application, the test area will be painted with 0.5 g of 10% sodium lauryl sulfate in vaseline after close-clipping in order to create a local irritation (if the test item is nonirritant).

Test Group

7±1 days after completion of the intradermal induction phase, a patch will be fully loaded with the test item extract. Then it will be applied to the test area and held in contact with the help of an occlusive dressing for 48 hours.

Control Group

7±1 days after completion of the intradermal induction phase, a patch will be fully loaded with the extraction medium. Then it will be applied to the test area and held in contact with the help of an occlusive dressing for 48 hours.

7.9. Challenge: Topical Application

The flanks of the treated and the control animals will be cleared of hair.

Test Group and Control Group

14±1 days after completion of the topical induction phase, a patch loaded with the test item extract will be applied to the left flank of the animals and a patch loaded with the extraction medium will be applied to the right flank (intraspecific control). The patches will be held in contact with the help of an occlusive dressing for 24 hours.

7.10. Clinical Observation

Test Group and Control Group

Approximately 21 hours after removing the patch, the challenge area will be cleaned and cleared of hair, if necessary. Approximately 24 hours after removing the patch the skin reaction will be observed and recorded according to the grades shown in Table 1. Further observations will be recorded 48 hours after patch removal.

Additionally all animals will be observed for signs of toxicity at least once daily during the test period.

Table 1: 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

7.11. Evaluation of Results

The percentage of sensitised animals in comparison to the control group will be calculated for each time of observation. The highest percentage will be used for the rating of the test item.

Individual data will be recorded in tabular form.

8. Reporting

The results of the study will be reported in a detailed report. The report will include the following:

- the copies of the GLP certificates
- the name and address of the sponsor, the study monitor and the test facility
- the study schedule
- the names of the study director, other scientists and supervisory personnel involved in the study
- the dates of all study plan amendments
- a list of all deviations from the study plan
- the Quality Assurance Statement, signed by the Quality Assurance Unit,
- the statement of compliance, signed by the study director
- information about the storage locations of the final report and related documents, specimens and samples
- the test item identification, either by name or code number
- the preparation of the test item
- the concentration, purity, stability, composition and other appropriate characteristics of the test item, if data are provided by the sponsor
- a description of the test system, including the biological materials used
- a detailed description of application and treatment, dose levels of the test item, toxicity data, negative and positive controls, historical laboratory data
- a description of the test animals: strain, health status, sex, weight at the beginning of the study, group size, animal husbandry
- a description of the reactions observed during the study
- data in tabular form
- a description of the methods with references
- a description, discussion and interpretation of all results
- a copy of the study plan (if required)

9. Distribution of the Study Plan

1 original (paper):	remains with the Study Director
2 copies (electronic or paper):	one to be sent to the Sponsor
	one to be sent to the QAU

10. References

10.1. Internal Eurofins Munich and BSL Munich SOPs

Standard Operating Procedures (SOPs), No. 11-3-1

10.2. Literature and Guidelines

[1] Chemikaliengesetz ("Chemicals Act") of the Federal Republic of Germany, Appendix 1 to § 19a as amended and promulgated on August 28, 2013 (BGBl. I S. 3498)

[2] Konsens-Dokument der Bund-Länder-Arbeitsgruppe Gute Laborpraxis ("Consensus Document of the National and Länder Working Party on Good Laboratory Practice") on the archiving and storage of records and materials, 5 May 1998.

[3] OECD Principles of Good Laboratory Practice (as revised in 1997); OECD Environmental Health and Safety Publications; Series on Principles of Good Laboratory Practice and Compliance Monitoring - Number 1. Environment Directorate, Organisation for Economic Co-operation and Development, Paris 1998

[4] Richtlinie 90/385/EWG des Rates vom 20. Juni 1990 zur Angleichung der Rechtsvorschriften der Mitgliedstaaten über aktive implantierbare medizinische Geräte, Amtsblatt Nr. L 189 vom 20/07/1990 S. 0017 - 0036

[5] Richtlinie 93/42/EWG des Rates vom 14. Juni 1993 über Medizinprodukte, Amtsblatt Nr. L 169 vom 12/07/1993 S. 0001 - 0043

[6] DIN EN ISO/IEC 17025: 2005: Allgemeine Anforderungen an die Kompetenz von Prüf- und Kalibrierlaboratorien, DIN Deutsches Institut für Normung e.V., Berlin, August 2005

[7] ISO 10993-1: 2009 "Biological evaluation of medical devices – Part 1: Evaluation and testing within a risk management process"

[8] ISO 10993-2: 2006 "Biological evaluation of medical devices – Part 2: Animal welfare requirements"

[9] ISO 10993-10: 2010 "Biological evaluation of medical devices – Part 10:Tests for irritation and skin sensitization"

[10] ISO 10993-12: 2012 "Biological evaluation of medical devices – Part 12: Sample preparation and reference materials"

[11] Directive 2010/63/EU of the European parliament of the council of 22 September 2010 on the protection of animals used for scientific purposes

[12] Deutsches Tierschutzgesetz (German Animal Welfare Act), 24. Juli 1972 (BGBl. I S. 1277)

[13] Magnusson B. and A. M. Kligman, (1969) The identification of contact allergens by animal assay. The guinea pig maximisation test. J. Invest. Dermatol., 52, 268

[14] Magnusson B. (1980), Identification of contact sensitisers by animal assay. Cont. Derm., 6, 46

[15] Robinson, M.K., Stotts, J., Danneman, PJ., Nusair, T.L., Bay, P.H.S. (1989), A risk assessment process for allergic contact sensitization. Fd Chem Toxicol 27, 479