

Study of Maximized Skin Sensitization for the Test Item

SMART PRINT BIO BITE SPLINT CLEAR

Report

REFERENCE METHOD: ISO 10993-10:2021

STUDY DIRECTOR: Andrea da Costa

DATE OF COMPLETION OF THE STUDY: November 11th, 2024

CONTRACT LABORATORY: **MEDLAB PRODUTOS DIAGNÓSTICOS LTDA.**
Octávio Teixeira Mendes Sobrinho Street, 35
Vila Santa Catarina - Zip Code: 04376-070
São Paulo, SP - Brazil

IDENTIFICATION: Study code: **BSDM2**

Study Number: **12922-1/2024.0**

SPONSOR: MMTECH PROJETOS TECNOLOGICOS
IMPORTACAO E EXPORTACAO LTDA
Rua Doutor Procopio Toletto Malta, 62
Zip Code: 13562-291 – São Carlos - SP – BRAZIL.

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

Study title: Study of Maximized Skin Sensitization for the Test Item SMART PRINT BIO BITE SPLINT CLEAR

Study number: 12922-1/2024.0

This study was conducted under my responsibility in accordance with NIT-DICLA-035 (INMETRO, Oct/19, Rev. 04) and its complementary documents, which meets the principles of Good Laboratory Practice as published by the OECD (Nº 1 [ENV/MC/CHEM (98) 17]).

This study was conducted in accordance with the study plan, approved by the Sponsor and Test Facility Manager and to the standard operating procedures. This report represents a true and accurate record of the results obtained. There were no major known circumstances that may have affected the quality or integrity of the study.

All original raw data, including electronic records, documentation, signed study plan, possible additions to the study plan, final report and test item rate will be retained in the GLP files of Medlab Produtos Diagnósticos Ltda.

Study Director
Medlab Produtos Diagnósticos Ltda

STATEMENT OF QUALITY ASSURANCE

Study title: Study of Maximized Skin Sensitization for the Test Item SMART PRINT BIO BITE SPLINT CLEAR

Study number: 12922-1/2024.0

Based on the Quality Assurance review, this final report was considered an accurate and true record of the data generated during the study.

This final report has been inspected for the respective study plan, standard operating procedure and raw data. Study procedures were monitored through process inspection.

The inspections were conducted in accordance with the standard operating procedures of the Quality Assurance of Medlab Produtos Diagnósticos Ltda.

Inspection dates and respective reporting dates to the Study Director and Test Facility Manager are presented below. These inspection reports are kept in the GLP files of Medlab Produtos Diagnósticos Ltda.

Inspection	Date of Inspection	Reporting dates	
		Study Director	Test Facility Manager
Study plan	August 30 th , 2024	August 30 th , 2024	August 30 th , 2024
Experimental phase*	June, 17 th and June, 21 st , 2024	July 05 th , 2024	July 05 th , 2024
Raw data	November 11 th , 2024	November 11 th , 2024	November 11 th , 2024
Final Report	November 11 th , 2024	November 11 th , 2024	November 11 th , 2024

* Process inspection performed at least every 12 months

Quality Assurance
Medlab Produtos Diagnósticos Ltda

GENERAL INFORMATION

Contributors

Andrea da Costa	Study Director
Roberta dos Santos Machado	Test Facility Manager
Paula Renata Bezerra	Quality Assurance
Emine Oshiro Sakaue	Quality Assurance
Suellen Karoline Moreira Bezerra	Technical Support
Paloma Oliveira	Technical Support
Mariucha Soares	Technical Support
Victoria Ferreira Cravo	Technical Support

Study dates

Study start date:	September 12 th , 2024
Experimental phase start:	September 20 th , 2024
Experimental phase end:	October 18 th , 2024
Study completion date:	November 11 th , 2024
English version:	November 11 th , 2024

Performing laboratory

This study was conducted at Medlab Produtos Diagnósticos Ltda, located at Rua Octávio Teixeira Mendes Sobrinho, 35 – CEP:04376-070, São Paulo – SP, Brazil.

Study plan adherence

No deviations from the study plan were recorded.

Amendment 01: In item 11.1 of the Study Plan, information about the steps of days 5-7 was not included: “*approximately 24 hours before induction by topical application, if there is no dermal irritation, the tested area (after being properly shaved) should be exposed to 0.5 mL of Sodium Lauryl Sulfate 10% to create local irritation. The remaining SDS will be removed before the 2nd induction*”. There is no impact on the study, since the experimental steps were followed according to the methodology and reference related to the Study.



Archives

All raw data and original study records are the property of the Sponsor. The data will be correctly registered, signed and kept at Medlab Produtos Diagnostics Ltda for five years. Upon completion of all studies, unused/damaged test items will be retained until the expiration date and then will be discarded.

INDEX

	PAGE
GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT	2
STATEMENT OF QUALITY ASSURANCE	3
GENERAL INFORMATION	4
1. ABSTRACT.....	8
2. INTRODUCTION.....	9
2.1. Study objective.....	9
2.2. Reference.....	9
2.3. Weight of evidence analysis.....	9
2.4. Animal welfare	9
3. MATERIAL AND METHOD	10
3.1. Test item**	10
3.2. Test system.....	11
3.3. Environmental conditions	12
3.4. Method of administration and reason for choice.....	12
3.5. Reference item (control).....	13
3.6. Material, reagents and equipment	13
3.7. Test item preparation.....	14
3.8. Experimental design.....	14
3.9. Results evaluation / Acceptance criterion	15
4. RESULTS.....	16
4.1. Body weight	16
4.2. Challenge evaluation	16
5. CONCLUSION	16
6. REFERENCES.....	16
TABLE 1: Individual body weight of the test system – polar extract.....	17
TABLE 2: Individual body weight of the test system – non-polar extract	18
TABLE 3: challenge evaluation – polar extract.....	19
TABLE 4: challenge evaluation – non-polar extract	20
TABLE 5: Incidence of positive reactions during challenge evaluation – polar extract	21
TABLE 6: Incidence of positive reactions during challenge evaluation – non-polar extract ..	21

APPENDIX 1	22
RECOGNITION CERTIFICATE OF COMPLIANCE WITH THE PRINCIPLES OF GOOD LABORATORY PRACTICES	22

1. ABSTRACT

The aim of this study was to evaluate the possible sensitizing effects of the test item **SMART PRINT BIO BITE SPLINT CLEAR** provided by MMTECH PROJETOS TECNOLOGICOS IMPORTACAO E EXPORTACAO LTDA. Skin sensitization is an immunologically mediated skin reaction to a substance, characterized in laboratory animals by the appearance of edema and erythema. The methodology used was ISO 10993-10 (2021).

For each extraction (polar and non-polar), ten guinea pigs (*Cavia porcellus*) had an immunological response induced through the intradermal injection of the test item in the scapula region, potentiated with the use of an adjuvant, followed by the topical application of the test item over the same location after a week apart. After the 14-day rest period, the animals were challenged with the topical application of the test item in the flank region. The control group of five animals was treated with vehicle during the induction period, and treated with the test item in the challenge period. The animals were weighed at the beginning and at the end of the experimental period, and clinically evaluated throughout the experimental period. No erythema and edema reactions were observed in the challenge assessment. According to the methodology adopted for the study, the test item **SMART PRINT BIO BITE SPLINT CLEAR** was considered non-sensitizing.

2. INTRODUCTION

2.1. Study objective

Skin sensitization is an immunologically mediated skin reaction to a substance, characterized in laboratory animals by the appearance of edema and erythema. The maximized method uses an adjuvant capable of stimulating the immune response, to enhance the sensitivity of the method. The present study aimed to evaluate the possible sensitizing effects of the test item **SMART PRINT BIO BITE SPLINT CLEAR**.

2.2. Reference

The study was conducted according to ISO 10993-10: Tests for skin sensitization, 2021.

2.3. Weight of evidence analysis

For reasons related to animal welfare, prior to conducting the study, an analysis of the evidence was performed, with available and relevant data from the test item. The testing strategy includes an assessment of human and/or animal data related to toxic effects. Test substance known to cause pain and discomfort due to corrosive or severely irritating properties need not be tested.

2.4. Animal welfare

Animals are maintained in the testing facility in accordance with local and international requirements outlined in the Standard Operating Procedures. Animals with ongoing signs of severe discomfort and/or pain at any stage of the study are humanely euthanized and the test item properly evaluated. Animal care procedures and decision criteria for euthanasia of moribund and severely distressed animals are described in detail in the Standard Operating Procedures.

3. MATERIAL AND METHOD

3.1. Test item**

Identification:	SMART PRINT BIO BITE SPLINT CLEAR
Receiving date at Medlab:	August 6 th , 2024
Category:	Medical device
Batch:	PBSF 007/24
Manufacturing date:	February 2024
Expiry date:	February, 2026
Active ingredient(s):	Not applied
CAS number of active ingredient(s):	Not applied
Declared composition:	Methacrylic Monomers >45% Oligomers< 50%; Dispersant <2%; Photo Initiator <2%; Additive <.01%; Pigments <1%
Physical state:	Solid
Other information:	Dimensions: 50x50x1.2mm Weight: 3.5g
Provided by:	MMTECH PROJETOS TECNOLOGICOS IMPORTACAO E EXPORTACAO LTDA.

**Information regarding the test item and/or reference item is the responsibility of the Sponsor.

3.2. Test system

Species:	<i>Cavia porcellus</i> (Guinea pigs)
Strain:	Dunkin-Hartley
Source:	Anilab, Paulínia-SP
Justification for the test system:	Guinea pigs are a species widely used in skin sensitization studies, and recommended in the test method
Number and sex:	15 males per extraction, 5 animals in the control group and 10 animals in the test group
Body weight:	Healthy young adults with body weight between 300 and 500 g
Date of birth:	August 11 th , 2024
Receiving date:	September 19 th , 2024
Acclimatization:	The animals were acclimated to laboratory conditions for 5 days before starting the test; animals with any signs of abnormality were not used in the study
Accommodation:	The animals were kept in conventional cages for the species during the acclimation and testing period, 5 animals per cage
Identification:	The test system was individually identified by marking with a felt-tip pen on the flank; the boxes were identified by labels containing the study number, lot of animals and dates of the experimental phase.

Feeding:

Commercial feed for the species (Qualy Nutrição Animal – Guinea pigs, batch 234-2, manufacture: 08/21/2024, validity: 12/19/2024) was supplied *ad libitum* during the acclimatization period; the feed is analyzed at each batch to verify the presence of microbiological contaminants. The feed provided did not show contamination that could affect the purpose or integrity of the study.

Water:

Filtered water was provided *ad libitum* in the acclimatization periods; the water is periodically analyzed for the presence of chemical and microbiological contaminants. The filtered water provided did not show contamination that could affect the purpose or integrity of the study.

3.3. Environmental conditions

The environmental conditions of the test room were monitored and recorded during the experimental period. The average temperature was 21.56°C, and the average relative humidity was 66.79 %. The animals were kept under automatic control of the 12/12 hours photoperiod.

3.4. Method of administration and reason for choice

The sample was applied intradermally (1st induction) and later topically in the dermis (2nd induction and challenge) of the test system as described in the methodology.

3.5. Reference item (control)

The vehicles used for the test item extraction were 0.9% sodium chloride solution (polar vehicle) and cottonseed oil (non-polar vehicle).

The quality assurance control of this assay is provided for in the reliability test carried out periodically (Periodic Positive Control). The last evaluation was carried out in September/2024, using 15 animals (5 animals in the control group and 10 animals in the test group), and the substance α -hexylcinnamaldehyde described in the table below was considered sensitizing (positive reactions grades 1 and 2 in test group animals).

Positive control: α – Hexylcinnamaldehyde
CAS number: 101-86-0
Batch: A4 30376-QA4
Manufacturing date: 09/08/2022
Expiry date: 06/07/2025

3.6. Material, reagents and equipment

Materials: Bottle with lid, gauze, syringe, sterile needle, felt-tip pen, hypoallergenic tape/adhesive tape, micropore, scissors, filter paper, cotton, cotton swab.

Reagents: 0.9% sodium chloride solution, cottonseed oil, 10% sodium dodecyl sulfate (SDS), liquid vaseline and complete Freund's adjuvant (FCA).

Equipment: Electronic scale, trimmer machines and incubator.

3.7. Test item preparation

Extraction of the test item was performed in accordance with ISO 10993-12 (2021). The test item was extracted at 50°C for 72 hours in an incubator in two vehicles (polar and non-polar) in a ratio of 3 cm² of test item to 1 mL of vehicle. The extracts were used within 24 hours of preparation.

Polar extraction:

Total prepared for 1st induction: 50 cm² of test item for 16.7 mL of 0.9% sodium chloride solution.

Total prepared for 2nd induction: 50 cm² of test item for 16.7 mL of 0.9% sodium chloride solution.

Total prepared for challenge: 50cm² of test item for 16.7 mL of 0.9% sodium chloride solution. The liquid resulting from this process (extract) presented a homogeneous and colorless appearance, as per the original color of the vehicle, without the presence of particulates, and was not submitted to any other additional process.

Non-polar extraction:

Total prepared for 1st induction: 50 cm² of test item for 16.7 mL of cottonseed oil.

Total prepared for 2nd induction: 50cm² of test item for 16.7 mL of cottonseed oil.

Total prepared for challenge: 50 cm² of test item for 16.7 mL of cottonseed oil.

The liquid resulting from this process (extract) presented a homogeneous and yellow appearance, according to the original color of the vehicle, without the presence of particulates, and was not submitted to any other additional process.

3.8. Experimental design

The test system was weighed before the beginning and at the end of the experiment. Trichotomy was performed approximately 24 hours before each application.

At the first induction (day 0), each animal received three pairs of intradermal injections (0.1 mL each injection) in the scapula region, with one injection of each pair being applied on each side of the midline, in a mirror fashion. The contents of the test group injections are described below:

- Injection 1: mixture 1:1(v/v) of FCA + 0.9% sodium chloride solution or cottonseed oil.
 - Injection 2: undiluted test item extract.
 - Injection 3: extract of the test item undiluted in a 1:1 ratio with the solution from injection 1
- The control group received three pairs of intradermal injections of 0.1 mL each, applied in the same sites as the animals in the test group, as described below:
- Injection 1: mixture 1:1 v/v of FCA + 0.9% sodium chloride solution or cottonseed oil.
 - Injection 2: 0.9% sodium chloride solution or undiluted cottonseed oil.
 - Injection 3: 0.9% sodium chloride solution or cottonseed oil in a 1:1 ratio with injection solution 1.

On day 6, the animals had the scapula area pretreated with 10% SDS massaged into the skin. On day 7, the animals received applications of filter paper soaked in 0.5 mL (0.25 mL on each side) of the test item extract (experimental group) and 0.5 mL (0.25 mL on each side) of cottonseed oil (control group) in the scapula region. The occlusive dressings remained in contact with the animals' skin for 48 hours, and after removal, the area was cleaned with a 0.9% sodium chloride solution.

On the 21st, the challenge period began. Animals (control and experimental groups) received cotton dressings soaked in 0.5 mL of undiluted test item extract. The occlusive dressing was kept in contact with the skin for 24 hours, and after removal, the area was cleaned with a 0.9% sodium chloride solution. Approximately 24 and 48 hours after dressing removal (48 and 72 hours after challenge exposure), skin reactions (erythema and edema) were assessed. Assessments were made according to the Magnusson and Kligman grading scale.

3.9. Results evaluation / Acceptance criterion

The criteria established for classifying the sample as a potential sensitizing agent were:

- a) Within 48 and/or 72 hours, 30% or more of the animals tested had a positive response (grade ≥ 1), in the absence of similar results in the control group.
- b) A positive reaction presented within 48 hours persistent at 72 hours in at least one animal, in the absence of similar results in the control group.

4. RESULTS

4.1. Body weight

Tables 1 and 2 show the initial and final body weight of the test systems, as well as the body weight variation. All animals showed weight gain at the end of the experimental period.

4.2. Challenge evaluation

Tables 3 and 4 show the evaluation of dermal reactions after challenge. Tables 5 and 6 show the incidence of positive reactions. Erythema and edema reactions were not observed for both polar and non-polar extracts.

5. CONCLUSION

According to the methodology adopted for the study, the test item **SMART PRINT BIO BITE SPLINT CLEAR** was considered non-sensitizing.

6. REFERENCES

INMETRO: NIT-DICLA-035 - Principles of Good Laboratory Practice – GLP, Rev. 04, October/2019 and its complementary documents.

ISO 10993:10 – Biological evaluation of medical devices. Part 10: Tests for skin sensitization, 2021.

ISO 10993:12 – Biological evaluation of medical devices. Part 12: Sample Preparation and Reference Materials, 2021.

OECD Environmental Health and Safety Publications, Series on Principles of Good Laboratory Practice and Compliance Monitoring. No. 1, 41p., Paris, 1998 (17).

TABLE 1: Individual body weight of the test system – polar extract

Test group	Body weight (g)		
	Initial	Final	Variation
01	300	450	150
02	306	384	78
03	318	404	86
04	326	440	114
05	388	554	166
06	324	442	118
07	320	444	124
08	376	486	110
09	324	390	66
10	368	490	122
Control group	Body weight (g)		
	Initial	Final	Variation
01	346	434	88
02	318	482	164
03	320	446	126
04	334	482	148
05	354	500	146

TABLE 2: Individual body weight of the test system – non-polar extract

Test group	Body weight (g)		
	Initial	Final	Variation
01	374	542	168
02	390	534	144
03	350	512	162
04	318	430	112
05	334	458	124
06	342	486	144
07	322	466	144
08	290	444	154
09	358	546	188
10	344	468	124
Control group	Body weight (g)138		
	Initial	Final	Variation
01	302	458	156
02	334	454	120
03	302	392	90
04	312	466	154
05	298	366	68

TABLE 3: Challenge evaluation – polar extract

Test group	Challenge evaluation	
	48 hours	72 hours
01	0	0
02	0	0
03	0	0
04	0	0
05	0	0
06	0	0
07	0	0
08	0	0
09	0	0
10	0	0
Control group	Challenge evaluation	
	48 hours	72 hours
01	0	0
02	0	0
03	0	0
04	0	0
05	0	0

0	No visible alteration
1	Discrete or Irregular Erythema
2	Moderate and Confluent Erythema
3	Intense Erythema and/or Edema

Magnusson and Kligman Scale (reactions ≥ 1 are considered positive)

TABLE 4: Challenge evaluation – non-polar extract

Test group	Challenge evaluation	
	48 hours	72 hours
01	0	0
02	0	0
03	0	0
04	0	0
05	0	0
06	0	0
07	0	0
08	0	0
09	0	0
10	0	0
Control group	Challenge evaluation	
	48 hours	72 hours
01	0	0
02	0	0
03	0	0
04	0	0
05	0	0

0	No visible alteration
1	Discrete or Irregular Erythema
2	Moderate and Confluent Erythema
3	Intense Erythema and/or Edema

Magnusson and Kligman Scale (reactions ≥ 1 are considered positive)

TABLE 5: Incidence of positive reactions during challenge evaluation – polar extract

Groups	48 hours	72 hours
Experimental	0/10 = 0%	0/10 = 0%
Control	0/5 = 0%	0/5 = 0%

TABLE 6: Incidence of positive reactions during challenge evaluation – non-polar extract

Groups	48 hours	72 hours
Experimental	0/10 = 0%	0/10 = 0%
Control	0/5 = 0%	0/5 = 0%

APPENDIX 1

RECOGNITION CERTIFICATE OF COMPLIANCE WITH THE PRINCIPLES OF GOOD LABORATORY PRACTICES

National Institute of Metrology, Quality and Technology – Inmetro General Coordination for Accreditation		
Statement of GLP Compliance		
GLP Recognition No. GLP BPL 0041		Initial Recognition: March 06 th , 2014
Medlab Produtos Diagnósticos Ltda. Rua Octavio Teixeira Mendes Sobrinho, 35 - Vila Santa Catarina - São Paulo – SP - Brasil		
<i>General Coordination for Accreditation of Inmetro grants to the above mentioned test facility the recognition of compliance with the OECD Principles of Good Laboratory Practice as part of the Brazilian GLP Monitoring Program to carry out non-clinical health and environmental safety studies, as described in the scope below:</i>		
Areas of expertise	Categories of Test Items	
Toxicity Studies, Efficacy Studies; Citotoxicity Studies	Pesticides, Their Components and Suchlike; Pharmaceutical Products; Veterinary Drugs; Sanitizers; Industrial Chemicals; Health Products; Medical Devices; Cosmetics; Food Additives	
Note: Categories of test items "pesticides", "pharmaceutical products", "cosmetics", "wood preservative", "feed additives", "veterinary drugs", "sanitizers", "industrial chemicals", "remedial for treatments of effluents and natural ecosystems" and "medical devices" are covered by Brazil's full adherence to the OECD Council Acts related to the Mutual Acceptance of Data (MAD) on Good Laboratory Practice.		
MARCOS VALERIO BARRADAS:66801095749 Assinado de forma digital por MARCOS VALERIO BARRADAS:66801095749 Dados: 2023.11.08 14:47:36 -03'00' MARCOS VALERIO BARRADAS General Coordinator for Accreditation Substitute		
<i>The current status of recognition must be checked on the email address http://www.inmetro.gov.br/monitoramento_BPL/certificados</i>		

MOD-CGCRE-027 – Rev. 09 – Apr. OUT/23 – Pg. 2/03