Study of Intracutaneous Reactivity for the Test Item

SMART PRINT BIO VITALITY

Final Report

REFERENCE METHOD: ISO 10993-23:2021

STUDY DIRECTOR: Fabiana de Oliveira Branchini

STUDY COMPLETION DATE: October 11th, 2024

PERFORMING LABORATORY:
MEDLAB PRODUTOS DIAGNÓSTICOS LTDA.
Rua Octávio Teixeira Mendes Sobrinho, 35
Vila Santa Catarina – CEP: 04376-070
São Paulo, SP - Brazil

IDENTIFICATION:
Study code: **BRII2**
Study number: **12917-1/2024.0**

SPONSOR:
MMTECH PROJETOS TECNOLÓGICOS IMPORTAÇÃO E EXPORTAÇÃO LTDA
Doutor Procópio Toledo Malta Street, 62
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GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

Study Title: Study of Intracutaneous Reactivity for the Test Item SMART PRINT BIO VITALITY

Study number: 12917-1/2024.0

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 (GLP) as published by the OECD (N° 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 obtained results. 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.

Fabiana de Oliveira Branchini
Analista de Toxicologia
CRBio: 68308/01-D

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Study Director
Medlab Produtos Diagnósticos Ltda

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STATEMENT OF QUALITY ASSURANCE

Study Title: Study of Intracutaneous Reactivity for the Test Item SMART PRINT BIO VITALITY

Study number: 12917-1/2024.0

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	08/30/2024	08/30/2024	08/30/2024
Experimental phase*	05/28 and 05/29/2024	07/04/2024	07/04/2024
Raw data	10/11/2024	10/11/2024	10/11/2024
Final Report	10/11/2024	10/11/2024	10/11/2024

* Process inspection performed at least annually

Emine Sakaue
Garantia da Qualidade

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Dados: 2024.10.11 09:10:54 -03'00'

Quality Assurance
Medlab Produtos Diagnósticos Ltda

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GENERAL INFORMATION

Contributors

Fabiana de Oliveira Branchini	Study Director
Roberta dos Santos Machado	Test Facility Manager
Emine Oshiro Sakaue	Quality Assurance
Suellen Karoline Moreira Bezerra	Technical support
Paloma Oliveira	Technical support
Fernanda Bolognese	Technical support

Study dates

Study start date:	September 10th, 2024
Experimental phase start:	September 13th, 2024
Experimental phase end:	September 20th, 2024
Study completion date:	October 11th, 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 amendments or deviations were registered to the study plan.

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 Diagnósticos Ltda for five years. The test item will not be retained until the expiration date, after which time it will be discarded.

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1. ABSTRACT

The aim of this study was to evaluate the local response to the test item **SMART PRINT BIO VITALITY** (provided by MMTECH PROJETOS TECNOLÓGICOS IMPORTAÇÃO E EXPORTAÇÃO LTDA.) through the intracutaneous application of the test item extract on the dorsum of rabbits (*Oryctolagus cuniculus*). The methodology used was ISO 10993-23 (2021).
Three adult, healthy, nulliparous and non-pregnant female rabbits were selected and maintained under controlled environmental conditions. The animals were shaved in the back region approximately 18 hours before application of the test item. Intracutaneous application of polar and non-polar extracts of the test item was performed in a volume of 0.2 mL, in 5 application sites in the test area, while in the control area the respective extraction vehicles were applied, under the same conditions as the extracts of the test item. The animals were weighed before application and at the end of the experimental period. The animals were evaluated for signs of erythema and edema 24, 48 and 72 hours after application of the test item. Reactions of erythema and edema grade 1 were observed only for non-polar extraction, for both test and control sites. The reactivity index was 0.0 for polar extraction and 0.0 for non-polar extraction. No systemic signs of toxicity were observed.
Under the conditions of the study, the test item **SMART PRINT BIO VITALITY** was considered in accordance with the adopted methodology.

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2. INTRODUCTION

2.1. Study objective

This study aimed to evaluate the local response to the test item **SMART PRINT BIO VITALITY** through the intracutaneous application of the test item extract on the dorsum of rabbits (*Oryctolagus cuniculus*).

2.2. Reference guideline

The study was performed according to ISO 10993-23: *Tests for skin irritation*, 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.

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3. MATERIAL AND METHOD

3.1. Test item[1]

Identification:	SMART PRINT BIO VITALITY
Received date at Medlab:	August 6th, 2024
Category:	Health Products
Batch:	PVA3 004/24
Manufacturing date:	April/2024
Expiration date:	April/2026
Active ingredient(s):	Not applicable
CAS number of the active ingredient(s):	Not applicable
Declared composition:	Amorphous Silica < 5%; Silanized Silica > 50%; Dispersant <4%; Photoinitiator <4%; Methacrylic Monomers >40%; Pigments <0.07%
Physical state:	Solid
Other information:	Dimensions: 50x50x1.2mm | Weight: 5g
Provided by:	MMTECH PROJETOS TECNOLÓGICOS IMPORTAÇÃO E EXPORTAÇÃO LTDA.

[1] Information provided by the Sponsor

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3.2. Test system

Species:	*Oryctolagus cuniculus* (rabbits)
Strain:	New Zealand
Source:	Anilab, Paulínia - SP
Justification for the test system:	Rabbits are a species widely used in skin irritation studies, and recommended in the test method
Number and sex:	3 nulliparous and non-pregnant females
Body weight:	Body weight above 2.0 kg
Date of birth:	02/03/2024 (391, 392 e 393)
Receiving date:	08/08/2024 (391, 392 e 393)
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
Identification:	The test system was individually identified by marking with a hydrographic pen on the ear; the cages were identified by labels containing the cage number and rabbit number
Feeding:	Commercial feed for the species (Qualy Nutrição Animal – Rabbits, batch 220-2, manufacture 08/07/2024, validity: 12/05/2024) was supplied *ad libitum* during the acclimatization period; the feed is analyzed at

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	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 20.5ºC, and average relative humidity was 72.9%. The animals were kept under automatic control of the 12/12 hours photoperiod.

3.4. Method of administration and reason for choice

The test item extract was applied intracutaneously on the dorsum 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 and cottonseed oil.

3.6. Material, reagents and equipment

Materials: Flask with lid, gauze, syringe and sterile needle.
Reagents: 0.9% sodium chloride solution and cottonseed oil.
Equipment: Electronic scale, trimmer machine and incubator.

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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 50ºC 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.

The test item has a surface area of 50 cm²

50 cm²/ 3 cm² = 16.6mL the vehicle

Total prepared for polar solution: 50 cm² (1 unit) of the test item to 16.6 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.

Total prepared for non-polar solution: 50 cm² (1 unit) of test item to 16.6 mL of vegetable 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

An area of approximately 10 x 15 cm from the dorsal region of the test system was shaved approximately 17 hours before the start of the test. After this process, the skin of the animals was evaluated, only those with intact skin and no lesions were used in the test. The test system was weighed at the beginning and end of the experiment.

Due to the nature of the test item and low toxicity risk, each extract was applied simultaneously to 3 animals. The pH of the polar extract was measured as 5.0. The extracts were applied intracutaneously in 5 different sites, in a volume of 0.2 mL per site, totaling 1.0 mL per animal. In the control areas, the same procedure was carried out using the extraction vehicles, 0.9% sodium chloride solution and vegetable oil.

The animals were evaluated for signs of erythema and edema, at periods of approximately 24, 48 and 72 hours after application, as well as for the occurrence of systemic clinical signs.

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3.9. Results evaluation / acceptance criterion

Animals were evaluated approximately 24, 48 and 72 hours after application of the test item for the presence of erythema and edema according to ISO 10993-23 (2021), and the degree of skin reactions was recorded according to the table below:

Erythema and eschar formation		Edema formation	
No erythema	0	No edema	0
Very slight erythema (barely perceptible)	1	Very slight edema (barely perceptible)	1
Well-defined erythema	2	Well-defined edema (well-defined edges)	2
Moderate erythema	3	Moderate edema (approximately 1mm)	3
Severe erythema (beef-redness) to eschar formation	4	Severe edema (more than 1mm and extending beyond exposure area)	4
Maximum score		**8**	

After a 72-hour evaluation, the erythema and edema totals for each animal were summed separately for test and control items and divided by 15 (3 evaluation periods x 5 application sites). The overall score for the test or control item was determined by summing the animal scores and dividing by the total number of animals. The intracutaneous reactivity index was obtained by subtracting the control score from the evaluated test item score.

The requirements for no intracutaneous reactivity are met if the intracutaneous reactivity index (difference between the mean test item score and the mean negative control score) is ≤ 1.0.

4. RESULTS

4.1. Mortality and clinical signs of toxicity-

No clinical signs of toxicity or mortality were observed among the animals.

4.2. Body weight

Table 1 shows the initial and final body weight of the test systems, as well as the body weight variation. At the end of experimental period all animals gained weight.

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4.3. Evaluation of erythema and edema reactions

Tables 2 to 4 show the record of erythema and edema reactions during the experimental period, as well as the calculation of the intracutaneous reactivity index. For polar extraction, no skin reactions were observed in animals and the intracutaneous reactivity index was 0.0. For non-polar extraction, reactions of erythema and edema grade 1 was observed for both test and control sites, and the intracutaneous reactivity index was 0.0.

5. CONCLUSION

Under the test conditions, the test item **SMART PRINT BIO VITALITY** was considered in accordance with the adopted methodology.

6. REFERENCES

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

ISO 10993:23 – Biological evaluation of medical devices. Part 23: Tests for irritation, 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).

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TABLE 1: Individual body weight (g) of the test system

Extraction	Animal	Initial weight	Final weight	Variation
Polar / Non-polar	**391**	3092	3116	24
	392	3264	3278	14
	393	3570	3596	26

TABLE 2: Scoring of intracutaneous reactions of the test system at 24-hour evaluation

Area	Animal	24 hours									
		Site 1		Site 2		Site 3		Site 4		Site 5	
		ER	ED	ER	ED	ER	ED	ER	ED	ER	ED
Control NaCl	**391**	0	0	0	0	0	0	0	0	0	0
	392	0	0	0	0	0	0	0	0	0	0
	393	0	0	0	0	0	0	0	0	0	0
Test NaCl	**391**	0	0	0	0	0	0	0	0	0	0
	392	0	0	0	0	0	0	0	0	0	0
	393	0	0	0	0	0	0	0	0	0	0
Control Vegetable oil	**391**	0	1	0	1	0	1	0	1	0	1
	392	0	1	0	1	0	1	0	1	0	1
	393	0	1	0	1	0	1	0	1	0	1
Test Vegetable oil	**391**	0	1	0	1	0	1	0	1	0	1
	392	0	1	0	1	0	1	0	1	0	1
	393	0	1	0	1	0	1	0	1	0	1

NaCl: 0.9% sodium chloride solution; ER: erythema; ED: edema

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TABLE 3: Scoring of intracutaneous reactions of the test system at 48-hour evaluation

Area	Animal	48 hours									
		Site 1		Site 2		Site 3		Site 4		Site 5	
		ER	ED	ER	ED	ER	ED	ER	ED	ER	ED
Control NaCl	391	0	0	0	0	0	0	0	0	0	0
	392	0	0	0	0	0	0	0	0	0	0
	393	0	0	0	0	0	0	0	0	0	0
Test NaCl	391	0	0	0	0	0	0	0	0	0	0
	392	0	0	0	0	0	0	0	0	0	0
	393	0	0	0	0	0	0	0	0	0	0
Control Vegetable oil	391	1	1	1	1	1	1	1	1	1	1
	392	0	1	0	1	0	1	0	1	0	1
	393	1	1	1	1	1	1	1	1	1	1
Test Vegetable oil	391	1	1	1	1	1	1	1	1	1	1
	392	0	1	0	1	0	1	0	1	0	1
	393	1	1	1	1	1	1	1	1	1	1

NaCl: 0.9% sodium chloride solution; ER: erythema; ED: edema

TABLE 4: Scoring of intracutaneous reactions of the test system at 72-hour evaluation and calculation of intracutaneous reaction index

Area	Animal	72 hours										Index calculation				
		Site 1		Site 2		Site 3		Site 4		Site 5		Total 1	Total 2	Total 3	Total 4	Index
		ER	ED	ER	ED	ER	ED	ER	ED	ER	ED					
Control NaCl	391	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	392	0	0	0	0	0	0	0	0	0	0	0	0			
	393	0	0	0	0	0	0	0	0	0	0	0	0			
Test NaCl	391	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	392	0	0	0	0	0	0	0	0	0	0	0	0			
	393	0	0	0	0	0	0	0	0	0	0	0	0			
Control Vegetable oil	391	1	1	1	1	1	1	1	1	1	1	25	1.66	3.98	1.32	0
	392	0	0	0	0	0	0	0	0	0	0	10	0.66			
	393	1	1	1	1	1	1	1	1	1	1	25	1.66			
Test Vegetable oil	391	1	1	1	1	1	1	1	1	1	1	25	1.66	3.98	1.32	
	392	0	1	0	1	0	1	0	1	0	1	10	0.66			
	393	1	1	1	1	1	1	1	1	1	1	25	1.66			

NaCl: 0.9% sodium chloride solution; ER: erythema; ED: edema
Total 1: sum of erythema and edema scores in all evaluations; Total 2: total 1 divided by 15; Total 3: sum of total 2 per area; Total 4: Total 3 divided by 3; Index: total 4 of test area minus total 4 of control area.

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APPENDIX 1 – CERTIFICATE OF RECOGNITION 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 06th, 2014

Medlab Produtos Diagnósticos Ltda.
Rua Octavio Teixeira Mendes Sobrinho, 35 - Vila Santa Catarina - São Paulo – SP - Brasil

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:

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.

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MARCOS VALERIO BARRADAS
General Coordinator for Accreditation Substitute

The current status of recognition must be checked on the email address http://www.inmetro.gov.br/monitoramento_BPL/certificados

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

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