Study of *in vivo* Pyrogen Test for the Test Item

SMART PRINT BIO VITALITY

Final Report

REFERENCE METHOD:	USP NF Online Chap.151 (last revision 2017)
STUDY DIRECTOR:	Andrea da Costa
STUDY COMPLETION DATE:	September 16th, 2024
ENGLISH VERSION:	September 16th, 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: **BPFA1** Study number: **12019-1/2024.0**
SPONSOR:	MMTECH PROJETOS TECNOLÓGICOS IMPORTAÇÃO E EXPORTAÇÃO LTDA Doutor Procópio Toledo Malta Street, 62 Morada dos Deuses - Zip code: 13.562-291 São Carlos - SP – Brazil

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GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

Study title: Study of *in vivo* Pyrogen Test for the Test Item SMART PRINT BIO VITALITY

Study number: 12019-1/2024.0

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.

Andrea da Costa, M.Sc, PhD
Diretor(a) de Estudos Toxicológicos *in vitro*
Supervisor(a) Laboratório de Toxicologia *in vitro* / *in vivo*
Bióloga CRBio/SP – 072416/01-D

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Study Director
Medlab Produtos Diagnósticos Ltda

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STATEMENT OF QUALITY ASSURANCE

Study title: Study of *in vivo* Pyrogen Test for the Test Item SMART PRINT BIO VITALITY

Study number: 12019-1/2024.0

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*	08/23/2024	09/05/2024	09/05/2024
Raw data	09/16/2024	09/16/2024	09/16/2024
Final Report	09/16/2024	09/16/2024	09/16/2024

* Inspection performed at least annually.

Emine Sakaue
Garantia da Qualidade

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Quality Assurance
Medlab Produtos Diagnósticos Ltda

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GENERAL INFORMATION

Contributors

Andrea da Costa	Study Director
Roberta dos Santos Machado	Test Facility Manager
Emine Oshiro Sakaue	Quality Assurance
Suellen Karoline Moreira Bezerra	Technical Support
Paloma Oliveira	Technical Support
Fabiana de Oliveira Branchini	Technical Support

Study Dates

Study start date:	September 6th, 2024
Experimental phase start:	September 7th, 2024
Experimental phase end:	September 11th, 2024
Study completion date:	September 16th, 2024
English version:	September 16th, 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 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. Test items will be held until the expiration date, after which it will be discarded.

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1. ABSTRACT

The aim of this study was to evaluate the febrile response to the test item **SMART PRINT BIO VITALITY** (provided by MMTECH PROJETOS TECNOLÓGICOS IMPORTAÇÃO E EXPORTAÇÃO LTDA.), through the intravenous injection of the extract of the test item in rabbits (*Oryctolagus cuniculus*). The method used was USP NF Online Chap.151 (last revision 2017).

Three adult, healthy, nulliparous and non-pregnant female rabbits were selected and maintained under controlled environmental conditions. The animals were placed in specific containers for the species and acclimatized for a minimum period of thirty minutes. After the acclimatization period, the control temperature of the animals was measured (base temperature for determining any temperature rise). The test solution (extract) was then applied intravenously. After application of the test solution, the temperature was again measured at intervals of approximately 30 minutes for a period of 3 hours. No animal showed a temperature increase equal to or greater than 0.5ºC.

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

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2. INTRODUCTION

2.1. Study objective

The aim of the present study was to evaluate the febrile response to the test item **SMART PRINT BIO VITALITY** through the intravenous injection of the extract of the test item in rabbits (*Oryctolagus cuniculus*).

2.2. Reference method

The study was performed according to the reference guideline USP NF Online Chap.151 (last revision 2017).

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 supplied 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 *in vivo* pyrogen studies, and recommended in the test method
Number and sex:	3 healthy, nulliparous and non-pregnant females
Body weight:	Body weight above 1.5 kg
Date of birth:	04/25/2024 (377, 378 and 383)
Receiving date:	07/18/2024 (377, 378 and 383)
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 period and were housed in specific restrainers for the species during the study.
Identification:	The test system was identified by marking with a hydrographic pen on the ear; 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)

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	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 recorded during the experimental period. The temperature ranged between 18.9°C and 20.1°C and the humidity ranged between 48.5% and 70.4%. During the acclimatization period, the animals were kept in a room with monitored temperature and humidity and automatic control of the 12/12 hours photoperiod.

3.4. Method of administration and reason for choice

The test item extract was injected intravenously into the marginal ear vein of the test system as described in the methodology.

3.5. Reference item

It was used 0.9% sodium chloride solution as extraction vehicle of the test item.

3.6. Material, reagents and equipment

Materials: Despyrogenized vial, micropore, sterile gauze, syringe and scalp.
Reagents: Pyrogen-free 0.9% sodium chloride solution.

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Equipment: Electronic scale, laminar flow, water bath, temperature probe and incubator.

3.7. Preparation of test item

The test item was extracted at 50ºC for 72 hours in an incubator, in the proportion of 3 cm^2 of the test item for 1 mL of 0.9% sodium chloride solution, according to ISO 10993-12 (2021). The test item has a surface area of 50 cm² (50 cm² * 7 units = 350 cm² / 3 cm² = 116.6 mL)

Total prepared: 350 cm² (7 units) of the test item for 116.6 ml of 0.9% sodium chloride solution.
The extract presented a homogeneous appearance, without the presence of particulates and was colorless, it was not submitted to any additional process after extraction, and it was used within 24 hours after preparation.

3.8. Experimental design

The animals were placed in specific containers for the species and acclimatized for a minimum period of thirty minutes. After the acclimatization period, the control temperature of the animals was measured (base temperature for determining any temperature rise). The test item extract was then applied as per the route and dose described above. After application of the test solution, the temperature was again measured at intervals of approximately 30 minutes for a period of 3 hours. The test system was observed during this study period for the presence of clinical changes and the measured temperatures were recorded.

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3.9. Results evaluation / acceptance criterion

Satisfactory: If no rabbit has an individual increase in temperature of 0.5ºC or more, above the respective temperature control, the product is within the requirements for the absence of pyrogen.

Retest: If one or more rabbits show an individual temperature increase of 0.5ºC or more, retest using another five rabbits.

Unsatisfactory: If more than three of the eight rabbits have an individual temperature increase of 0.5ºC or more, and if the sum of the eight individual temperature increases exceeds 3.3ºC, the product under test is not within the requirements for the absence of pyrogen.

Note: Consider any decrease in temperature as zero.

4. RESULTS

4.1. Clinical signs and temperature measurements

No clinical changes were observed in the animals during the experimental period. No animal showed an individual increase in temperature equal to or greater than 0.5°C.

Table 1 shows body weight, application volume and temperature variations.

TABLE 1 – Body weight, volume injected, and temperature measurements of the animals treated with the extract of **SMART PRINT BIO VITALITY**

Rabbit number	Body weight (g)	Volume injected (mL)	Temperature readings (minutes)							Temperature variation
			Control	30	60	90	120	150	180	
377	3450	34.5	39.0	39.4	39.3	39.3	39.3	39.1	39.2	0.4
378	3498	35.0	39.4	39.5	39.5	39.5	39.5	39.4	39.4	0.1
383	3332	33.3	39.1	39.4	39.3	39.3	39.3	39.3	39.3	0.3
									Total raises	0.8ºC

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5. CONCLUSION

Under the test conditions, the test **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 10993:12 – 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).

USP NF Online Cap.151 (last revision 2017) - Pyrogen test.

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