Nelson Labs
A Sotera Health company

Sponsor:
Daniel Membreno
Infinita Lab Inc.
Balentine Dr. Suite 200
Newark, CA 94560

Pyrogenicity Test in New Zealand White Rabbits - ISO/USP

Test Article:	Cured coating Panels
Purchase Order:	ZB-PO-6839
Study Number:	1570812-S01
Study Received Date:	05 Dec 2022
Testing Facility:	GV Research Platform c/o Palamur BioSciences
Deviations:	None

Summary: Enclosed is the final report for the testing we coordinated for you. The information is retained by the testing laboratory.

If you have any questions, please feel free to call or email any of our Subcontracting personnel at 801-290-7500 or biocompservice@nelsonlabs.com. Thank you for testing with Nelson Laboratories, LLC.

Tanner Welch electronically approved — Reviewed By: Tanner Welch

08 Feb 2023 20:58 (+00:00) — Study Completion Date and Time

STUDY REPORT

STUDY NUMBER

23109

STUDY TITLE

Material Mediated Pyrogenicity Test with Cured coating panels
in New Zealand White Rabbits

TEST GUIDELINE

ISO 10993-11:2017

ISO 10993-12:2021

USP Chapter-151

SPONSOR

NELSON LABORATORIES

6280 S. Redwood Road,

Salt Lake City, UT 84123, USA.

CRO

GV RESEARCH PLATFORM PVT LTD.,

Sy. No. 403/1 (Old), 120 (New), 4th Floor, Niharika Jubilee One,

Road No.1, Jubilee Hills, Hyderabad – 500033, Telangana State, India.

TEST FACILITY

PALAMUR BIOSCIENCES PRIVATE LIMITED

SH-20, Karvina, Madigattla Village, Bhoothpur Mandal,

Mahabubnagar– 509 382, Telangana (India).

Study Director: Mr. Adapa Satish Kumar, M.Pharm.

Study Completion Date: 07.02.2023

STUDY DIRECTOR'S STATEMENT

Study Number : 23109

Study Title : Material Mediated Pyrogenicity Test with Cured coating panels in New Zealand White Rabbits.

I hereby declare that this study was performed in accordance with mutually agreed and approved study plan that was constructed based on the Standard Operating Procedures of Palamur Biosciences Private Limited (Test Facility). Palamur Biosciences Private Limited complies with various national/international quality systems such as OECD GLP, AAALAC, CDSCO, NABL/ISO-17025.

The report is a complete, true and accurate representation of the study, it reflects the raw data generated during the study period, as mentioned in the approved Study Plan.

As a Study Director, I accept overall responsibility for the technical conduct of the study as well as the documentation, analysis, interpretation and reporting of the results and validity of the data.

All the documents pertaining to the study, including the raw data, original study plan and final report have been retained at the archives of the test facility.

Study Director : Mr. Adapa Satish Kumar, M.Pharm.

Signature :

Date : 07.02.2023

Palamur Biosciences Private Limited,
SH-20, Karvina, Madigattla Village, Bhoothpur Mandal,
Mahabubnagar – 509 382, Telangana (India).

CERTIFICATE OF AFFIRMATION

Study Number : 23109

Study Title : Material Mediated Pyrogenicity Test with Cured coating panels in New Zealand White Rabbits

This is to certify that the test facility management has provided sufficient number of qualified personnel, appropriate facilities, equipment and materials in timely manner and proper conduct of this study in accordance with mutually agreed study plan.

Test Facility Management : **Dr. S. Ramamoorthy, Ph.D., F.A.Sc.**

Signature :

Date : 07.02.2023

Palamur Biosciences Private Limited

CONTENTS

ABBREVIATIONS

^{0}C	:	Degree Celsius
%	:	Percentage
cm	:	Centimetre(s)
CPCSEA	:	Committee for the Purpose of Control and Supervision of Experiments on Animals
CCSEA	:	Committee for Control and Supervision of Experiments on Animals
GLP	:	Good Laboratory Practice
IAEC	:	Institutional Animal Ethics Committee
ISO	:	International Organization for Standardization
Kg	:	Kilogram(s)
min	:	Minute(s)
mL	:	Millilitre(s)
mm	:	Millimetre(s)
NF	:	National Formulary
No.	:	Number
OECD	:	Organization for Economic Co-operation and Development
PBS	:	Palamur Biosciences Private Limited
QAU	:	Quality Assurance Unit
RO	:	Reverse Osmosis
SOP	:	Standard Operating Procedure
SP	:	Study Plan
TFM	:	Test Facility Management
TIDS	:	Test Item Data Sheet
USP	:	United States Pharmacopeia

MATERIAL MEDIATED PYROGENICITY TEST WITH CURED COATING PANELS IN NEW ZEALAND WHITE RABBITS

SUMMARY AND CONCLUSION

Pyrogenicity test with Cured coating Panels, sponsored by **NELSON LABORATORIES**, was tested in New Zealand White rabbits at Palamur Biosciences Private Limited. This study was conducted as per the agreed study plan constructed as per USP, ISO 10993-12:2021 and ISO 10993-11:2017.

Three rabbits were selected and fasted overnight with free access to water. The animals were administered with extract of Cured coating Panels. 614.08 cm^2 of test item was extracted using 102 mL of physiological saline (0.9% sodium chloride injection). Each animal was administered intravenously at 10 mL/Kg b.w. The temperature readings were recorded at 30, 0 minutes before administration, and at 30, 60, 90, 120, 150, 180 minutes post administration. All the animals were normal and did not show more rise in individual temperature (0.5°C).

Conclusion

Based on the above results, the given test item, Cured coating Panels pass the Pyrogen test and concluded as **non-pyrogenic** when administered to New Zealand White rabbits under the experimental conditions of the study.

1. STUDY OBJECTIVE

The purpose of this pyrogen test was to identify any pyrogen/ foreign substance that produce a febrile / feverish response from the test item extract of Cured coating Panels when injected intravenously to the animals.

2. STUDY COMPLIANCE

- This study was conducted as per the mutually agreed study plan and the Standard Operating Procedures of Palamur Biosciences Private Limited.

3. TEST GUIDELINES

- Biological Evaluation of Medical Devices - Part 11: Tests for systemic toxicity (ISO 10993-11:2017).
- Biological Evaluation of Medical Devices - Part 12: Sample preparation and reference materials (ISO 10993-12:2021).
- United States Pharmacopeia (USP): Biological Tests- Pyrogen Test, Chapter 151.

4. STUDY SCHEDULE

Study Initiation Date	:	10.01.2023
Experiment Start Date	:	28.01.2023
Acclimatization Start Date	:	28.01.2023
Administration of test item extracts	:	03.02.2023
Experiment Completion Date	:	03.02.2023
Study Completion Date	:	07.02.2023

5. LIST OF STUDY PERSONNELS (RESPONSIBILITIES)

Personnel	Name
Study Director	Mr. Adapa Satish Kumar, M.Pharm.
Study Personnel	Mr. A. Sri Rama Charyulu, M.Sc. Mr. A. Levin Astley, M.Sc.

6. MATERIALS AND METHODS

6.1 Test item details

Date of receipt at test facility: 10 January 2023

The following information is provided by the Sponsor. A copy of the TIDS was attached as **APPENDIX-1** and will be included in the Final report.

Name of the test item	:	Cured coating Panels
Physical appearance	:	Black rectangles
Lot No.	:	NA
Intended use	:	NA
Storage condition	:	Ambient (18 to 28°C)
Sterility	:	Non-Sterile
Sponsor's study code	:	1570812
Contact duration	:	Limited (≤ 24 hr)
Surface area	:	614.08 cm^2
Extraction Ratio	:	6 cm^2/mL
Extraction condition	:	50±2°C and 72±2 hrs
Date of Expiry	:	NA
Supplied by	:	Nelson Laboratories 6280 S. Redwood Road, Salt Lake City, UT 84123 USA

Safety of handling: Protective gloves, face mask, aprons/ protective suit and goggles were used to ensure the health and safety of the personnel.

Test item identity and stability: The identity and stability of the test item is the responsibility of the sponsor. No analysis was performed to confirm at Palamur Biosciences Private Limited.

6.2 Solvent details

Polar Solvent

Name of the Solvent	:	Sodium chloride injection IP 0.9% W/V
Batch No.	:	2H21087

Appearance	:	Clear colourless solution
Manufacturing date	:	Aug 2022
Expiry date	:	Jul 2025

7. TEST SYSTEM

7.1 Justification for the selection of test system

Rabbits were chosen as the test system because this species is commonly used for pyrogen test and it meets the regulatory requirement for most of the regulatory agencies.

7.2 Test system details

Species	:	*Oryctolagus cuniculus* (Rabbit)
Strain	:	New Zealand White
Source	:	Palamur Biosciences Private Limited, Mahabubnagar, Telangana, India.
Body weight at the time of dosing	:	1531.46 - 1648.35g
Sex	:	Young adult healthy male
Number of animals	:	5
Route of administration	:	Intravenous
Acclimatization period	:	Prior to acclimatization, a physical health examination was performed on all animals by a veterinarian. Animals with any evidence of ill health or poor physical condition were not used. Healthy animals were acclimatized for 6 days.

7.3 Housing

Location	:	Rodent experiment facility, Room No. 6, Block A
Temperature	:	19.6 – 22.8°C
Relative Humidity	:	46-60 %
Photo period	:	12 hours light and 12 hours dark
Room air exchanges	:	Minimum 12-15 air exchanges per hour
Caging	:	Animals were housed individually in stainless steel rabbit cages (Approximately internal dimensions of 580 mm x 580 mm x 500 mm)

with corn cob bedding.

Method of identification	:	All the animal cages were identified by cage cards and followed by corresponding individual animal numbers marked with marker pen on the ear pinna of each rabbit.
Diet and water	:	Rabbit pellet diet manufactured by Krishna Valley Agro LLP. The source of the water was borewell water which was purified with RO water plant present at the premises. Both drinking water and feed were provided *ad libitum*.

Note: The feed and water were routinely analyzed and are considered not to contain any contaminants that could reasonably be expected to affect the purpose or integrity of the study. The water and feed analysis reports were maintained in the study raw data file.

8. ANIMAL WELFARE

The test facility is certified by the Committee for Control and Supervision of Experiment on Animals (CCSEA) for breeding and experimentation. Certification No.: 1312/PO/RcBiBt-S/RcBiBt-L/09/CPCSEA.

9. IAEC approval

This study has been approved by the Institutional Animals Ethics Committee (IAEC) of the Palamur Biosciences Private Limited, Proposal No.: PAL/IAEC/2023/01/01/24 under the Project Title “Material Mediated Pyrogenicity Test with Cured coating panels in New Zealand White Rabbits” dated on 05.01.2023. All the procedures were in compliance with the guidelines of CCSEA, India. An authorized photocopy of IAEC approval was maintained in the study raw data file.

10. EXPERIMENTAL PROCEDURES

10.1 Fasting of animals

One day before inserting the rectal temperature probes (preliminary test and initial test), the animals were fasted overnight with free access to water.

10.2 Pre-Selection of animals

Initially a preliminary test was conducted with 5 animals for 2 days during acclimatization phase. The animals were restrained and each probe from digital telethermometer was inserted into individual rabbit's rectum. The temperature readings were monitored (after 1 hr from the time of probe insertion) and recorded for 3 hr with every 30 min interval time.

10.3 Selection of animals

Based on the temperature readings from 5 animals, three animals were selected for the test and fasted overnight before administration of extract.

10.4 Preparation of test item extract

As per the TIDS provided by the sponsor, no absorption check was performed.

614.08 cm^2 of test item was extracted with,102 ml of physiological saline (Extraction ratio 6cm^2/mL) at 51°C for 71 hrs 45 min.

After extraction, the extract was collected in a sterile glass beaker. The polar test item extracts was changed to Orange- brown coloured particulate suspension and the pH of extract was found as 6. The extract was pre-warmed at 37 °C for 30 minutes under aseptic condition prior to administration. All the glassware were sterilized and used.

10.5 Administration of extract

The animals were restrained 60 min before administration. Each thermometer probe was lubricated and inserted into each rabbit's rectum to a depth of 7.5 cm and the temperatures were recorded.

The initial temperature of each animal was recorded. The pre-warmed extract was taken in a sterile syringe fitted with a 24 G needle IV infusion set and administered to each animal intravenously at 10 mL/kg body weight within 6-7 min. The temperatures were recorded prior to administration.

11. OBSERVATIONS

11.1 Mortality / viability

All animals were examined individually twice daily for mortality throughout the experiment period.

11.2 Clinical observations

All animals were examined once daily for any signs of toxicity during acclimatization and experimental phase.

11.3 Body weight

Individual body weights of the animals were recorded on the day of acclimatization and on the day of administration of test item extract.

11.4 Recording of temperature

During acclimatization, the temperature probes were inserted 1 hr before and the temperatures were recorded at -60, -30, 0, 30, 60, 90, 120, 150, 180 min, where -60, -30 readings were not considered.

On the day of administration (experiment), the individual animal temperatures were recorded at -30 minutes, 0 minute (prior to administration), and post administration at 30, 60, 90, 120, 150, 180 min. The 30 min post administration reading was not considered for evaluation.

12. RESULTS

12.1 Mortality / viability

No mortality was observed in any of the animals used in this experiment (**Refer Table-21.1**).

12.2 Clinical observations

All animals were normal and no signs of clinical toxicity were observed in any of the animals used in this experiment.

12.3 Body weight

All animals showed increase in body weight and within normal weight range limit (**Refer Table-21.2**).

12.4 Recording of temperature

All animals temperature neither varied more than 1°C nor exceed 39.8°C during the preliminary test.

On experimental day, the individual animal temperatures were recorded at -30 min, 0 min (before administration), and post administration at 30, 60, 90, 120, 150, 180 min (**Refer Table-21.3**). The temperature reading at 30 min post administration was not considered for evaluation.

12.5 Evaluation of results

The initial temperature reading (0 minute before administration) of each animal is the control temperature of that individual animal. Post administration, the individual body temperature did not increase 0.5°C or more when compared to its control temperature. (**Refer Table-21.3**).

13. FATE OF ANIMAL

After completion of experiment, the animals were returned to the animal house in-charge.

14. CONCLUSION

Based on the results obtained in the present study, it is concluded that the given test item, Cured coating Panels pass the Pyrogen test and concluded as **non-pyrogenic** when administered to New Zealand White rabbits under the experimental conditions of the study.

15. ARCHIVES

Raw data and other documents arising out of this study will be stored in the archives of Palamur Biosciences Private Limited, SH-20, Karvina, Madigattla Village, Bhoothpur Mandal, Mahabubnagar – 509382, Telangana (India), for up to nine years after completion of the study work. No later than nine years after completion of the study, instructions for returning or disposing of the archives will be requested from the Sponsor. Implementation of such instructions may be at charge to the Sponsor.

The archived materials will include the following documents:

- Study plan (Original 1 of 2)
- All relevant correspondence concerned with the study.
- Raw data

- Study report (Original 1 of 2)

16. STUDY PLAN AMENDMENTS

No study plan amendment was made during the Study period.

17. DEVIATIONS

No deviations were observed during the experiment.

18. REFERENCES

- Compendium of CCSEA 2018: Guidelines for Laboratory Animal Facility 2015: 7; pg- 61-96.
- Biological Evaluation of Medical Devices - Part 1, Evaluation and Testing within a Risk Management Process (ISO 10993-1:2018).
- Biological Evaluation of Medical Devices - Part 2: Animal welfare requirements. ISO 10993-2:2006 (E).
- Indian Pharmacopeia 2014.
- Biological Evaluation of Medical Devices - Part 11: Tests for systemic toxicity, ISO 10993-11:2017 (E).
- United States Pharmacopeia (USP 40): Biological Tests- Pyrogen Test, Chapter 151.

19. SPONSOR REPRESENTATIVE (Monitoring Scientist)

Sponsor's Representative: Tanner Welch

Monitoring Scientist: Chad Summers

Nelson Laboratories

6280 S. Redwood Road,

Salt Lake City, UT 84123, USA.

20. CONFIDENTIAL

Information, data embodied in this study report are strictly confidential and are issued on the understanding that they will be held confidentially and not disclosed to third parties without the prior consent of the Sponsor.

21. TABLES

21.1 Mortality

Animal Number		1 to 5	01 to 03
Sex		Female	Female
Day of Observations	Mortality	Incidences	Observations
Acclimatization Period	Mortality	0/5	-
Experiment Day (Treatment)	Mortality	-	0/5

21.2 Body weight and administration details

Body weight				**Administration details**	
A.No.	**Acclimatization start day (g)**	**A.No.**	**Treatment day (g)**	**Volume injected (mL)**	**Time taken to inject (min)**
1	1619.24	01	1648.35	16.5 (17)	7
2	1503.94	02	1531.46	15.3 (15)	6
3	1512.70	03	1540.82	15.4 (15)	7
4	1519.32	-	-	-	-
5	1523.35	-	-	-	-

Key: g- grams; min- minutes; mL- millilitres. A.No. 1, 2 & 3 were selected for main study and marked as 01, 02 & 03.

21.3 Temperature readings – Main test

A.No	Temperature Readings (°C)										
	Before administration of test item extract		Initial	After administration of test item extract						Max.	Maximum individual rise
	-30 min#	0 min#	0 min*	30 min#	60 min	90 min	120 min	150 min	180 min		
01	38.4	38.3	38.3	38.5	38.4	38.5	38.6	38.5	38.4	38.6	0.3
02	38.6	38.4	38.4	38.6	38.5	38.4	38.5	38.5	38.6	38.6	0.2
03	38.5	38.4	38.4	38.6	38.6	38.7	38.6	38.5	38.6	38.7	0.3

Key: *Temperature recorded prior to dosing; #- Not considered for evaluation; A.No.- Animal number; mL- millilitres; °C- degree Celsius; min- minutes; Max.- Maximum.

APPENDIX-1: TEST ITEM DATA SHEET

Test / Reference Item Data Sheet (Medical Device) Palamur bio

Study Sponsor (Company Name and address)	Nelson Laboratories 6280 S. Redwood Road Salt Lake City, UT 84123 USA
Study compliance	☐ GLP ■ NON GLP
Name of Test Item / (Sponsor's ID for Test / Reference Item)	Cured coating Panels
Name of Reference Item	n/a
Intended Use of the Device	n/a
Contact Duration	■ Limited (≤ 24 hr) ☐ Prolonged (> 24 hr to 30 Days) ☐ Permanent (> 30 Days)
Total Surface area / Dimensions (length, Inner Diameter, Outer Diameter, width, radius etc.)	614.08cm2
Extraction Ratio	☐ 3cm²/mL ■ 6cm²/mL ☐ 0.2g/mL ☐ 0.1g/mL
Weight (For irregular shaped devices)	☐ GVRP/Palamur to measure weight during testing
Physical appearance	black rectangles
Batch No / Product Code	n/a
Lot No.	n/a
Extraction Conditions: ☐ 37 ± 1°C and 24 ± 2 hrs ☐ 37 ± 1°C and 72 ± 2 hrs ■ 50 ± 2°C and 72 ± 2 hrs ☐ 70 ± 2°C and 24 ± 2 hrs ☐ 121 ± 2°C and 1 ± 0.1hrs ☐ Others:	
Sterility: ☐ Sterile ■ Non-sterile	
Absorption check	☐ Yes ■ No
If Absorption check not required, provide the details	non-absorbant
Date of Expiry / Valid up to	n/a
Quantity sent	2
Component (s) to be used for extraction / testing	☐ Refer to provided Nelson Aux document for additional details
Predicate device (for Implantation / others) (if supplied by Sponsor kindly fill the Reference Item Data Sheet)	☐ Supplied by Sponsor ☐ Procured by Test facility ■ n/a

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10/01/23
VERIFIED BY

APPENDIX-1: TEST ITEM DATA SHEET (Continued)

Test / Reference Item Data Sheet (Medical Device) Palamur bio

Storage conditions: ■ Ambient (18 to 28°C) ☐ Cool and dry (2 to 8°C) ☐ Frozen (<-10°C) ☐ Others _____ (specify the temperature / storage condition)	
Safety Precautions, if any	n/a
Material safety data sheet attached	☐ Yes ■ No
Certificate of analysis	☐ Yes ■ No
Others if any,	
Fate of remaining Test/Reference Item after completion of projects	☐ Dispose at test facility ■ Return back ☐ Return unused samples
List of Studies to be performed	☐ Guinea Pig Maximization Sponsor Study Code: ☐ Intracutaneous Reactivity Sponsor Study Code: ☐ Acute Systemic Toxicity Sponsor Study Code: ■ Material Mediate Pyrogenicity Sponsor Study Code: 1570812
Any additional information (optional)	
Name of the Sponsor's Representative	Tanner Welch
Name of the Monitoring Scientist	Chad Summers
Signature and date	29 Dec 2022

PALAMUR BIOSCIENCES PRIVATE LIMITED
SH-20, Karvina, Madigattla Village, Bhoothpur Mandal, Mahabubnagar District,
Telangana State -509382, India.
Mobile: +91 9000411835, 7382448855 EXT:201

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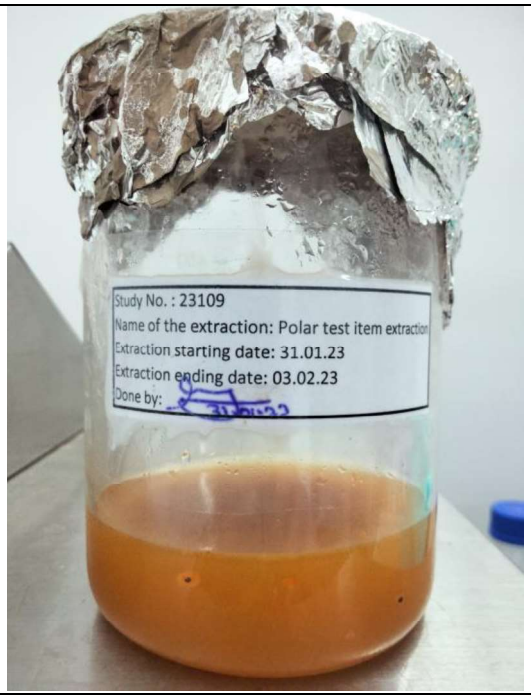
VERIFIED BY QC

APPENDIX-2: REPRESENTATIVE IMAGES

	
Polar test before extraction	Polar test after extraction