Nelson Labs
A Sotera Health company

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

Intracutaneous Reactivity Test in New Zealand White Rabbits - ISO

Test Article:	Cured coating Panels
Purchase Order:	ZB-PO-6839
Study Number:	1570808-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 — 08 Feb 2023 20:52 (+00:00)
Reviewed By: Tanner Welch — Study Completion Date and Time

STUDY REPORT

STUDY NUMBER

23110

STUDY TITLE

Intracutaneous reactivity test with Cured coating Panels in New Zealand White Rabbits

TEST GUIDELINE

ISO 10993-23:2021

ISO 10993-12:2021

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 : 23110

Study Title : Intracutaneous Reactivity 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 : 23110

Study Title : Intracutaneous Reactivity 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,

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ABBREVIATIONS

AAALAC	:	Association for Assessment and Accreditation of Laboratory Animal Care
^{0}C	:	Degree Celsius
%	:	Percentage
b.w.	:	Body Weight
w/v	:	weight/volume
cm	:	Centimetre
cm^2	:	Centimetres Square
CFR	:	Code of Federal Regulation
CPCSEA	:	Committee for the Purpose of Control and Supervision of Experiments on Animals
CCSEA	:	Committee for Control and Supervision of Experiments on Animals
g	:	Gram(s)
GLP	:	Good Laboratory Practice
hr	:	Hour(s)
IAEC	:	Institutional Animal Ethics Committee
ISO	:	International Organization for Standardization
Kg	:	Kilogram(s)
mg	:	Milligram(s)
min	:	Minute(s)
mL	:	Millilitre(s)
mm	:	Millimetre(s)
No.	:	Number
OECD	:	Organization for Economic Co-operation and Development
PAL	:	Palamur
PBS	:	Palamur Biosciences Private Limited
RO	:	Reverse Osmosis
SOP	:	Standard Operating Procedure
SR	:	Study Report

TOX	:	Toxicology
TFM	:	Test Facility Management
<	:	Less than

INTRACUTANEOUS REACTIVITY TEST WITH CURED COATING PANELS IN NEW ZEALAND WHITE RABBITS

SUMMARY AND CONCLUSION

Intracutaneous Reactivity Test with Cured coating Panels extracts in New Zealand White Rabbits sponsored by **Nelson Laboratories** was tested at Palamur Biosciences Private Limited. The intracutaneous reactivity test was performed as per ISO 10993-23:2021- Biological Evaluation of Medical Devices - Part 23: Tests for Irritation, ISO 10993-12:2021 - Biological Evaluation of Medical Devices - Part 12: Sample preparation and reference materials, in compliance with mutually agreed study plan.

The test item was extracted in the ratio of 6cm^2/mL at 51°C for 71 hrs 55 min. with physiological saline (polar extract) and sesame oil (non-polar extract), separately. Both, polar and non-polar controls (without test item) were also exposed to similar conditions.

The test was performed with 3 rabbits by injecting the prepared polar and non-polar extracts of control & test item intracutaneously at the fur clipped area (approximately, 10 X 15 cm^2). The animals were observed for clinical signs of toxicity and mortality throughout the experiment. All the animal's body weight was recorded on day 0 and after completion of experiment (Day 3). The skin reactions for erythema and oedema were observed and scored at 30 min., 24, 48 and 72 hr following the intracutaneous injections.

All animals were normal and no signs of clinical toxicity were observed throughout the experiment. All the animals showed increase in body weight. The mean skin reaction score for polar and non-polar extracts of test item was 0.0 and 0.0, respectively.

Conclusion

Based on the results obtained, it is concluded that the polar and non-polar extracts of the test item, Cured coating Panels meets the requirement of ISO 10993-23:2021 and is classified as **Non-irritant** for polar solvent and non-polar solvent extracts when injected intracutaneously to the rabbit skin under the conditions of present study.

1. STUDY OBJECTIVE

The purpose of this intracutaneous reactivity test was to identify the irritation potential of the test item Cured coating Panels extracts when administered intracutaneously to the skin of New Zealand White rabbits.

2. STUDY COMPLIANCE

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

3. TEST GUIDELINES

- Biological Evaluation of Medical Devices - Part 23: Tests for skin irritation (ISO 10993-23:2021).
- Biological Evaluation of Medical Devices - Part 12: Sample preparation and reference materials (ISO 10993-12:2021).

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
Euthanasia	:	06.02.2023
Experiment Completion Date	:	06.02.2023
Study Completion Date	:	06.02.2023

5. LIST OF STUDY PERSONNEL (RESPONSIBILITIES)

Personnel	Name
Study Director	Mr. Adapa Satish Kumar, M.Pharm.
Study Personnel	Mr. A. Sri Rama Charyulu, M.Sc. Mr. Levin Astley, M.Sc. Ms. Rajitha, B.Sc., MLT.
Study Pathologist	Dr. P. V. Sai Charitha, M.V.Sc.

6. MATERIALS AND METHODS

6.1 Test item details

Date of receipt at test facility: 10 January 2023

The following information was provided by the Sponsor. A copy of the TIDS was attached as **APPENDIX-2.**

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	:	1570808
Contact duration	:	Limited (≤ 24 hr)
Surface area	:	231.04 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

Non-Polar Solvent

Name of the Solvent	:	Sesame oil
Batch No.	:	372322
Appearance	:	Clear Yellow colour liquid
Manufacturing date	:	05.2022
Expiry date	:	05.2025

Note: Physical appearance of the solvents before extraction were considered as normal.

7. TEST SYSTEM

7.1 Justification for the selection of test system

Rabbits are the standard laboratory species used for assessment of irritation potential of the test item recommended globally by many regulatory authorities. New Zealand White strain was used due to availability of literature.

7.2 Test system details

Species	:	*Oryctolagus cuniculus* (Rabbit)
Strain	:	New Zealand White
Source	:	Palamur Biosciences Private Limited, Mahabubnagar, Telangana State, India.
Body weight at the time of dosing	:	2296.33 - 2525.49g
Age	:	Healthy young adult
Sex	:	Male
Number of animals	:	3

Acclimatization	:	Prior to acclimatization, a physical health examination was performed on all animals by the veterinarian. Healthy animals were acclimatized to the experimental room 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 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

This study will be performed at PBS which is approved by CCSEA with registration number 1312/PO/RcBiBt-S/RcBiBt-L/09/CPCSEA and AAALAC.

9. IAEC APPROVALS

This study has been approved by the Institutional Animals Ethics Committee (IAEC) of the Test Facility, IAEC protocol No. **PAL/IAEC/2023/01/01/32** under the Project Title " Intracutaneous Reactivity Study with Cured coating Panels in New Zealand White Rabbits" dated on 05.01.2023. An authorized photocopy of IAEC approval was maintained in the study raw data file.

10. EXPERIMENTAL PROCEDURES

10.1 Route of Administration and Justification for Selection

Control and test item extracts were administered intracutaneously as per the ISO 10993 Part 23 standard specifications.

10.2 Dose Selection and Justification for Selection

As suggested in the guideline ISO 10993, Part 23:2021, the undiluted extracts were used for the testing.

10.3 Preparation of test and control items

As per the TIDS provided by the sponsor, absorption check was not performed.

The test item was extracted in the ratio 6cm^2/mL at 50°C for 71 hrs 55 min. with polar (physiological saline) and non-polar solvents (sesame oil), separately.

Both, polar and non-polar controls were also kept under similar conditions. After extraction, the test item(s) were removed from the respective extracts. No colour change and no particulates were observed in both the polar and non- polar control extracts, whereas polar test item extracts was changed to Orange- brown coloured particulate suspension and non-polar extract was changed to dark yellow coloured suspension. Both extracts were found normal. Representative images were attached as an **Appendix 3.**

Name of the Extraction	Surface area of the test item (cm^2)	Volume of solvent added (mL)	Physical appearance		Volume of Extract (mL)	pH of the extract
			Before extraction	After extraction		
Polar control extraction	-	10	Normal	No colour change	10	7
Non-polar control extraction	-	10	Normal	No colour change	10	6
Polar test item extraction	231.04	38.5 = 39 mL	Normal	Orange-brown coloured particulate suspension	38	6
Non-polar test item extraction	231.04	38.5 = 39 mL	Normal	Dark yellow coloured suspension	38	5

Note: g-gram, mL- millilitre.

10.4 Initial considerations

The pH of the control & test item extracts was found to be approximately, 6.0 for polar and 5.0 for non-polar extracts which is between pH 2.0 and pH 11.5. No centrifugation and filtration were performed and the extracts were administered without dilution to the test system.

10.5 Preparation of animals

16 hr before the test, the animal's fur was clipped closely on the dorsal surface, both sides of the spinal cord (approximately, 10 x 15 cm^2) on each rabbit by using clipper. Care was taken to avoid abrading the skin. All animals were found with healthy intact skin.

10.6 Administration of extracts

To each animal, a total of 20 injections with a volume of 0.2 mL per injection of the extract were injected intracutaneously to four sites on the fur clipped area. To each site, 5 injections of respective extract were injected (**Refer Figure-1**). The injected sites were marked with a non-irritant permanent marker to identify the sites.

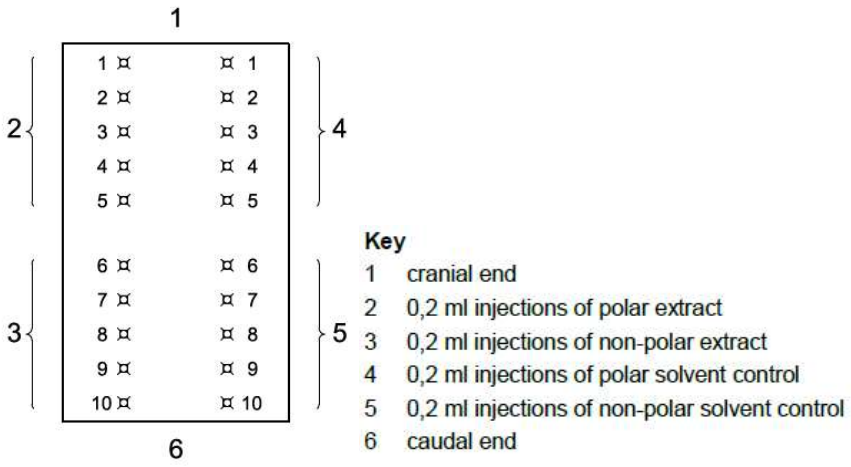

Figure 1: Arrangement of Injection Sites

11. OBSERVATIONS

11.1 Mortality / viability

All animals were examined individually twice daily for mortality during experimental period.

11.2 Clinical signs observations

All animals were observed once daily for any clinical signs of toxicity during experimental period.

11.3 Body weight

Individual body weight of the animals was recorded at the start of acclimatization, prior to the induction phase (Day 0) and on completion of the experiment (Day 3).

11.4 Grading of skin reactions

The skin responses were observed immediately after injection (within 30 min), 24, 48 and 72 hr following the intradermal injections. The skin reactions were graded and recorded according to the grades given in **APPENDIX-1**.

11.5 Evaluation of test results

The sum of erythema and oedema grades 24, 48 and 72 hr for individual animal were determined for each site (test and control). To calculate the score of a test or control site on each individual animal, each total was divided by 15 (3 scoring time points × 5 test or control injection sites). The overall mean score for each test and corresponding control sites was determined by adding the scores of the three animals and divided by three. The

final test item extract score was obtained by subtracting the score of the control extract score from the test extract score.

12. PATHOLOGY

12.1 Euthanasia

All animals were humanely sacrificed by carbon dioxide asphyxiation at termination and discarded.

12.2 Necropsy

As there was no mortality was observed during the experiment period, gross necropsy was not performed.

13. RESULTS

13.1 Mortality

No mortality was observed in any of the animals used in this experiment (**refer Table-23.1**).

13.2 Clinical signs

All animals were normal and did not show any signs of clinical toxicity during experiment (**refer Table-23.2**).

13.3 Body weights

All animals showed increased body weight when compared with Day-0 (**refer Table-23.3**).

13.4 Grading of skin reactions

The skin reactions for erythema and oedema grades were recorded with in 30 min. at 24, 48 and 72 hr. The individual animal grading of skin reactions was recorded (**refer Table-23.5**).

The difference of the mean skin reaction scores for polar and non-polar extracts of test item was 0.0 and 0.0, respectively (**refer Table-23.6**).

14. POSITIVE CONTROL TEST (RELIABILITY CHECK)

The positive control has not been included in this study, as the reliability of the intracutaneous test was tested separately for the positive control using the Sodium Lauryl Sulphate. The latest positive control test result (**Study No.: 22626, conducted on June-July 2022**) was attached as **Annexure -1**.

15. CONCLUSION

Based on the above results obtained, it is concluded that the polar and non-polar extracts of the test item, Cured coating Panels meets the requirement of ISO 10993-23:2021 and is classified as **Non-irritant** for polar solvent and non-polar solvent extract when injected intracutaneously to the rabbit skin as per the experimental conditions.

16. ARCHIVES

Raw data and other documents arising out of the study will be stored in the archive of Palamur Biosciences Private Limited, SH-20, Karvina, Madigattla Village, Bhoothpur Mandal, Mahabubnagar, Telangana, India for up to one year after completion of the study. No later than one year 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 additional charge to the sponsor. No data will be discarded without the Sponsor's written consent.

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).

17. STUDY PLAN AMENDMENT

No Study plan amendment was made during the Study period (Appendix-4).

18. DEVIATIONS

No study plan and SOP deviations were observed during the Study.

19. DATA COMPILATION

The data produced during the experimental period were tabulated in the raw data file and included in the study report.

20. 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 12: Sample preparation and reference materials (ISO 10993-12:2021).
- Biological Evaluation of Medical Devices - Part 2: Animal welfare requirements (ISO 10993-2:2022).
- Biological Evaluation of Medical Devices - Part 23: Tests for irritation (ISO 10993-23:2021).

21. 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.

22. 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.

23. TABLES

23.1 Mortality

Animal Number		1 to 3	01 to 03
Sex		**Male**	**Male**
Day of Observations*	Mortality	Incidences	Incidences
Acclimatization Period (Day 1-6)	Mortality	0/3	-
Treatment / Experiment Phase (Day 0-3)	Mortality	-	0/3

*Mortality was observed twice daily.

23.2 Clinical signs

Animal Number		1 to 3	01 to 03
Sex		**Male**	**Male**
Day of Observations*	Clinical Signs	Incidences	Incidences
Acclimatization Period (Day 1 to 6)	Normal	3/3	-
Treatment / Experiment Phase (Day 0-3)	Normal	-	3/3

*Clinical signs were observed once daily.

23.3 Body weight (g)

A.No.	Sex	Acclimatization	Pre-treatment (Day 0)	Pre sacrifice (Day 3)	% Body weight change (Day 0- Day 3)
01	Male	2510.69	2525.49	2546.81	0.84
02		2428.47	2445.65	2462.54	0.69
03		2277.70	2296.33	2311.46	0.66

Note: A.No.- Animal number; g- grams.

$$\text{Calculation for \% body weight change} = \frac{\text{Body weight of pre sacrifice} - \text{Body weight of pre treatment}}{\text{Body weight of pre treatment}} \times 100$$

23.4 Administration details

A.No.	Injection site	Sample	Volume per injection (mL)	Number of injections/ site
01	Left cranial end Right cranial end Left caudal end Right caudal end	Polar test item extract Polar control extract Non-polar test item extract Non-polar control extract	0.2	5
02	Left cranial end Right cranial end Left caudal end Right caudal end	Polar test item extract Polar control extract Non-polar test item extract Non-polar control extract	0.2	5
03	Left cranial end Right cranial end Left caudal end Right caudal end	Polar test item extract Polar control extract Non-polar test item extract Non-polar control extract	0.2	5

Note: A.No.- Animal number.

23.5 Individual grading of skin reactions

Animal Number	Observation Time	Site	Polar extract				Non-Polar extract			
			Control		Test		Control		Test	
			E	O	E	O	E	O	E	O
01	24 hours	1	0	0	0	0	0	0	0	0
		2	0	0	0	0	0	0	0	0
		3	0	0	0	0	0	0	0	0
		4	0	0	0	0	0	0	0	0
		5	0	0	0	0	0	0	0	0
	48 hours	1	0	0	0	0	0	0	0	0
		2	0	0	0	0	0	0	0	0
		3	0	0	0	0	0	0	0	0
		4	0	0	0	0	0	0	0	0
		5	0	0	0	0	0	0	0	0
	72 hours	1	0	0	0	0	0	0	0	0
		2	0	0	0	0	0	0	0	0
		3	0	0	0	0	0	0	0	0
		4	0	0	0	0	0	0	0	0
		5	0	0	0	0	0	0	0	0

Note: E- Erythema and eschar formation; O- Oedema; 0- No erythema/ oedema.

Individual grading of skin reactions (Continued)

Animal Number	Observation Time	Site	Polar extract				Non-Polar extract			
			Control		Test		Control		Test	
			E	O	E	O	E	O	E	O
02	24 hours	1	0	0	0	0	0	0	0	0
		2	0	0	0	0	0	0	0	0
		3	0	0	0	0	0	0	0	0
		4	0	0	0	0	0	0	0	0
		5	0	0	0	0	0	0	0	0
	48 hours	1	0	0	0	0	0	0	0	0
		2	0	0	0	0	0	0	0	0
		3	0	0	0	0	0	0	0	0
		4	0	0	0	0	0	0	0	0
		5	0	0	0	0	0	0	0	0
	72 hours	1	0	0	0	0	0	0	0	0
		2	0	0	0	0	0	0	0	0
		3	0	0	0	0	0	0	0	0
		4	0	0	0	0	0	0	0	0
		5	0	0	0	0	0	0	0	0

Note: E- Erythema and eschar formation; O- Oedema; 0- No erythema/ oedema.

Individual grading of skin reactions (Continued)

Animal Number	Observation Time	Site	Polar extract				Non-Polar extract			
			Control		Test		Control		Test	
			E	O	E	O	E	O	E	O
03	24 hours	1	0	0	0	0	0	0	0	0
		2	0	0	0	0	0	0	0	0
		3	0	0	0	0	0	0	0	0
		4	0	0	0	0	0	0	0	0
		5	0	0	0	0	0	0	0	0
	48 hours	1	0	0	0	0	0	0	0	0
		2	0	0	0	0	0	0	0	0
		3	0	0	0	0	0	0	0	0
		4	0	0	0	0	0	0	0	0
		5	0	0	0	0	0	0	0	0
	72 hours	1	0	0	0	0	0	0	0	0
		2	0	0	0	0	0	0	0	0
		3	0	0	0	0	0	0	0	0
		4	0	0	0	0	0	0	0	0
		5	0	0	0	0	0	0	0	0

Note: E- Erythema and eschar formation; O- Oedema; 0- No erythema/ oedema.

23.6 Total and mean score of skin reactions

Animal Number	Polar extract				Non-polar extract			
	Control		Test		Control		Test	
	E	O	E	O	E	O	E	O
01	0	0	0	0	0	0	0	0
02	0	0	0	0	0	0	0	0
03	0	0	0	0	0	0	0	0
Total Grade	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Score (Total grade/15)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
No. of animals observed per sample	3	3	3	3	3	3	3	3
Mean individual score	0	0	0	0	0	0	0	0
Mean reaction score (E+O)	**0.0**		**0.0**		**0.0**		**0.0**	
Final Score (T-C)	**0.0**				**0.0**			

Note: E- Erythema and eschar formation; O- Oedema, T-Test, C- Control.

ANNEXURE-1: RELIABILITY CHECK

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Partnering Research

INTRACUTANEOUS REACTIVITY TEST WITH SODIUM LAURYL SULPHATE (SLS) IN NEW ZEALAND WHITE RABBITS

SUMMARY AND CONCLUSION

Intracutaneous Reactivity Test with Sodium Lauryl Sulphate (SLS) in New Zealand White Rabbits was tested at Palamur Biosciences Private Limited. The intracutaneous reactivity test was performed as per ISO 10993-23:2021- Biological Evaluation of Medical Devices - Part 23: Tests for Irritation, OECD principles on Good Laboratory Practice.

10% of test item (Sodium Lauryl Sulphate (SLS)) was prepared using distilled water. Distilled water was used as control item.

The test was performed with 3 rabbits by injecting intracutaneously the prepared test & control item to the fur clipped area (approximately, 10 X 15 cm^2). The animals were observed for clinical signs of toxicity and mortality throughout the experiment. All the animals' body weight recorded on day 0 and after completion of experiment. The skin reactions for erythema and oedema were observed and scored at 24, 48 and 72 hr following the intracutaneous injections.

All animals were normal throughout the experiment. All the animals exhibited very slight erythema (barely perceptible), well defined erythema and moderate erythema during the 24 hr and 48 hr and get normalized by 72 hrs observation on the test item administered site. No erythema & oedema was observed at control site. All the animals showed increase in body weight. The mean skin reaction score for test item (Sodium Lauryl Sulphate (SLS)) and control was 1.05 and 0.0, respectively.

Conclusion

Based on the results obtained, it is concluded that the Test Item, Sodium Lauryl Sulphate (SLS) does not meets the requirement of ISO 10993-23:2021 and is classified as **Irritant** to the rabbit skin when injected intracutaneously as per the experimental conditions.

ANNEXURE-1: RELIABILITY CHECK (Continued)

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21.6 Individual grading of skin reactions

Animal Number	Observation Time	Site	Control item		Test item	
			E	O	E	O
01	30 min.	1	0	0	2	1
		2	0	0	1	2
		3	0	0	2	1
		4	0	0	2	1
		5	0	0	1	1
	24 hours	1	0	0	2	0
		2	0	0	1	1
		3	0	0	2	1
		4	0	0	1	1
		5	0	0	1	1
	48 hours	1	0	0	1	0
		2	0	0	0	0
		3	0	0	1	0
		4	0	0	0	0
		5	0	0	1	0
	72 hours	1	0	0	0	0
		2	0	0	0	0
		3	0	0	0	0
		4	0	0	0	0
		5	0	0	0	0

Note: E- Erythema and eschar formation; O- Oedema; 0- No erythema/ oedema; 1- very slight erythema/ Very slight oedema (barely perceptible); 2- Well defined erythema/ Slight oedema (edges of area well defined by definite raising).

ANNEXURE-1: RELIABILITY CHECK (Continued)

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Individual grading of skin reactions (Continued)

Animal Number	Observation Time	Site	Control item		Test item	
			E	O	E	O
02	30 min.	1	0	0	2	1
		2	0	0	2	2
		3	0	0	1	2
		4	0	0	2	1
		5	0	0	2	2
	24 hours	1	0	0	2	1
		2	0	0	1	1
		3	0	0	1	1
		4	0	0	2	1
		5	0	0	2	1
	48 hours	1	0	0	1	0
		2	0	0	0	0
		3	0	0	1	0
		4	0	0	1	0
		5	0	0	0	0
	72 hours	1	0	0	0	0
		2	0	0	0	0
		3	0	0	0	0
		4	0	0	0	0
		5	0	0	0	0

Note: E- Erythema and eschar formation; O- Oedema; 0- No erythema/ oedema; 1- very slight erythema/ Very slight oedema (barely perceptible); 2- Well defined erythema/ Slight oedema (edges of area well defined by definite raising).

ANNEXURE-1: RELIABILITY CHECK (Continued)

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Individual grading of skin reactions (Continued)

Animal Number	Observation Time	Site	Control item		Test item	
			E	O	E	O
03	30 min.	1	0	0	2	2
		2	0	0	1	2
		3	0	0	1	2
		4	0	0	2	1
		5	0	0	1	2
	24 hours	1	0	0	2	1
		2	0	0	1	1
		3	0	0	1	1
		4	0	0	2	1
		5	0	0	1	2
	48 hours	1	0	0	1	0
		2	0	0	0	0
		3	0	0	1	0
		4	0	0	1	0
		5	0	0	0	1
	72 hours	1	0	0	0	0
		2	0	0	0	0
		3	0	0	0	0
		4	0	0	0	0
		5	0	0	0	0

Note: E- Erythema and eschar formation; O- Oedema; 0- No erythema/ oedema; 1- very slight erythema/ Very slight oedema (barely perceptible); 2- Well defined erythema/ Slight oedema (edges of area well defined by definite raising).

ANNEXURE-1: RELIABILITY CHECK (Continued)

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21.7 Total and mean score of skin reactions

Animal Number	Control item		Test item	
	E	O	E	O
01	0	0	10	4
02	0	0	11	5
03	0	0	10	7
Total Grade	0	0	31	16
Score (Total grade/15)	0.0	0.0	2.07	1.07
No. of animals observed per sample	3	3	3	3
Mean individual score	0.0	0.0	0.69	0.36
Mean reaction score (E+O)	**0.0**		**1.05**	

Note: E- Erythema and eschar formation; O- Oedema.

APPENDIX-1: GRADING OF SKIN REACTIONS

Erythema and Eschar Formation:

No erythema	0
Very slight erythema (barely perceptible)	1
Well defined erythema	2
Moderate erythema	3
Severe erythema (beef redness) to eschar formation preventing grading of erythema	4

Maximum possible: 4

Oedema Formation:

No oedema	0
Very slight oedema (barely perceptible)	1
Slight oedema (edges of area well defined by definite raising)	2
Moderate oedema (raised approximately 1 mm)	3
Severe oedema (raised more than 1 mm and extending beyond area of exposure)	4

Maximum possible: 4

Total possible score for irritation: 8

APPENDIX-2: TEST ITEM DATA SHEET (TIDS)

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.)	231.04cm2
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	4
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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APPENDIX-2: TEST ITEM DATA SHEET (TIDS) (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: 1570808 ■ Acute Systemic Toxicity Sponsor Study Code: 1570810 ☐ Material Mediate Pyrogenicity Sponsor Study Code:
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

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10/01/23
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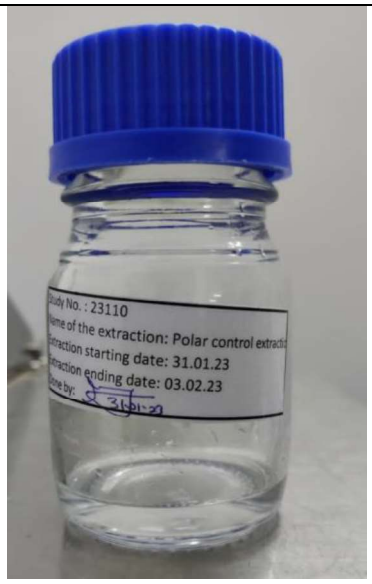
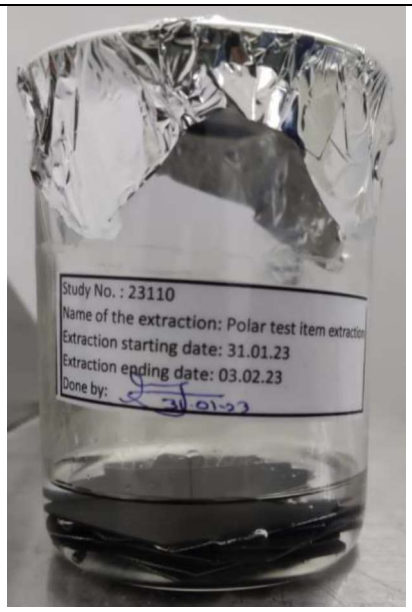
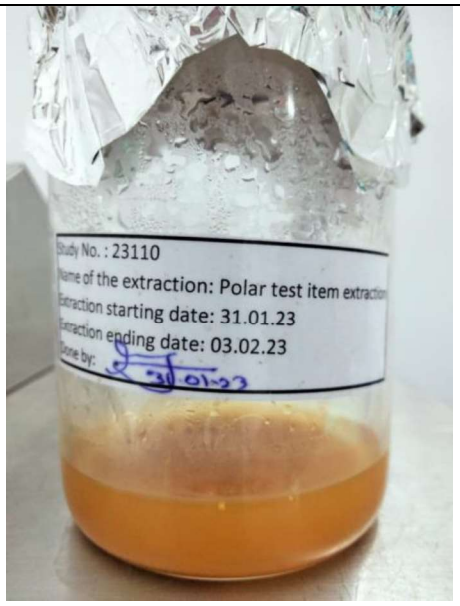
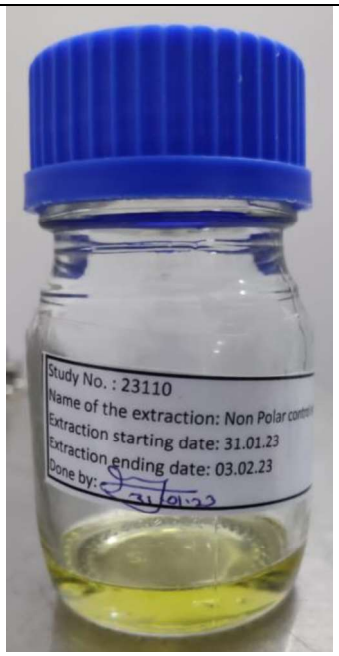
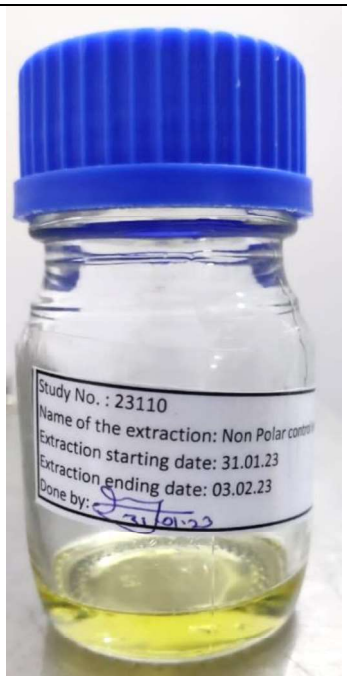
APPENDIX-3: REPRESENTATIVE IMAGES

	
Polar control before extraction	Polar control after extraction
	
Polar test before extraction	Polar test after extraction

APPENDIX-3: REPRESENTATIVE IMAGES (Continued)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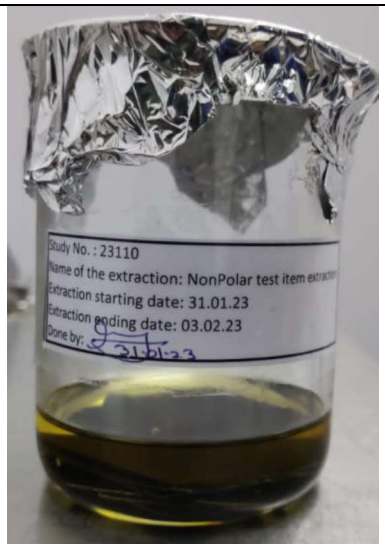	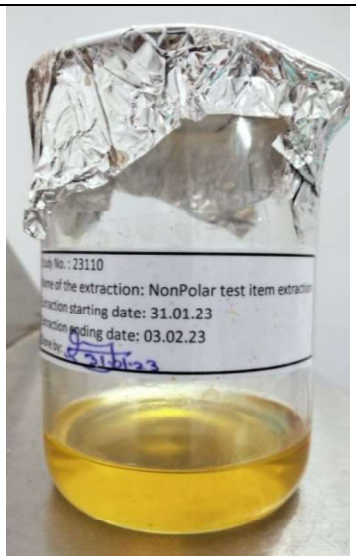
Non-Polar test before extraction	Non-Polar test after extraction