NELSON LABORATORIES

Sponsor:
Rose Cattolico
University of Washington
P.O. Box 355640
Seattle, WA 98195

ISO/USP Intracutaneous Reactivity Irritation Test in Rabbits

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

Test Article:	DLE-14-48-2
Nelson Laboratory Number:	864524
Testing Lab:	Sinclair Research

Results: The test article (device) did not cause skin irritation under the conditions of this assay.

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

Subcontracting Project Coordinator Jennifer Shaw, B.S.

27 Jan 2016
Date

sinclair research

ACCREDITED
Biological Testing
Cert #3221.01

FINAL REPORT

STUDY TITLE:	**ISO/USP Intracutaneous Reactivity Irritation Test in Rabbits**
PROTOCOL NUMBER:	D10989 (Version 2)
STUDY NUMBER:	D10989.046-454
TEST ARTICLE NAME:	DLE-14-48-2
TEST ARTICLE LOT NO:	N/A
TEST FACILITY:	Sinclair Research Center (SRC), LLC. (AAALAC Accredited) 562 State Road DD Auxvasse, MO 65231, USA Phone: (573) 387-4400 Fax: (573) 387-4404
SPONSOR:	Nelson Laboratories, Inc. 6280 South Redwood Road Salt Lake City, UT 84123
NELSON REFERENCE NO:	864524
DATA REQUIREMENTS:	GLP
DATE SAMPLE RECEIVED:	29-Dec-2015
STUDY INITIATION DATE:	29-Dec-2015
STUDY COMPLETION DATE:	26-Jan-2016
RESULTS SUMMARY:	The test article (device) did not cause skin irritation under the conditions of this assay.

QUALITY ASSURANCE UNIT SUMMARY

The study has been performed under Good Laboratory Practice regulations (FDA, 21 CFR, Part 58 - Good Laboratory Practice for Non-clinical Laboratory Studies) and in accordance with standard operating procedures and study protocol. The quality assurance unit inspected this study on the dates listed below. The report accurately reflects the raw data.

Phase Inspected	Date Inspected	Date Reported to Study Director & Management
Protocol (Version 2)	31-Jul-2014	31-Jul-2014
72 Hour Dermal Score	08-Jan-2016	08-Jan-2016
Draft Report and Data	25-Jan-2016	25-Jan-2016
Final Report	26-Jan-2016	26-Jan-2016

Quality Assurance Auditor: Ashley Abernathy Date 26Jan16

Ashley Abernathy

GOOD LABORATORY PRACTICES STATEMENT

The SRC study referenced in this report was conducted in compliance with Good Laboratory Practice (GLP) Regulations set forth in Title 21 Part 58 of the Code of Federal Regulations of the United States of America. Other portions of this study that were not performed by or under the direction of SRC, including the characterization and stability testing of the test article (device), were the responsibility of the sponsor and are exempt from this GLP statement.

Study Director: Rachael Mullins Date 26Jan16

Rachael Mullins, B.S., Scientist I

KEY STUDY PERSONNEL

Rachael Mullins, B.S.	Study Director
Susan Schnapp, RVT, ALAT	Test Facility Management
Chris Hanks, DVM, M.S., DACLAM	Senior Staff Veterinarian
Catherine Selby, M.S.	Director of Operations
Karen Curtis	Quality Control Manager

OBJECTIVE

The objective of this study was to determine if the test article (device) produced an irritation reaction when injected intracutaneously in rabbits as required of the medical device biocompatibility testing identified in ISO 10993 Part 10.

TEST ARTICLE IDENTIFICATION

Test Article Name:	DLE-14-48-2
Lot/Batch #:	N/A
Nelson Reference No.:	864524
SRC Test Article ID #:	8559
Sterile/Non-sterile:	Non-Sterile
Storage Conditions:	Room temperature
Intended Use/Application:	Unknown
Description:	yellow liquid
MSDS/CofA:	N/A

CONTROL ARTICLE IDENTIFICATION

Test Article Name:	Buffer solution
Lot/Batch #:	N/A
SRC Test Article ID #:	8560
Sterile/Non-sterile:	Non-Sterile
Storage Conditions:	Room temperature
Intended Use/Application:	Unknown
Description:	clear liquid
MSDS/CofA:	N/A

TEST ARTICLE CHARACTERIZATION

The sponsor was responsible for all test article characterization data as specified in the GLP regulations. The identity, strength, stability, purity, and chemical composition of the test article were solely the responsibility of the sponsor. It was the responsibility of the sponsor to ensure that the test article submitted for testing was representative of the final product that was subjected to materials characterization.

EXPERIMENTAL DESIGN

Three animals were used for this study. The test article and control article were intracutaneously injected at five sites on each side of the rabbits. The appearance of each injection site was noted immediately after injection. Each site was graded for tissue reaction (erythema and edema) at 24 ± 2, 48 ± 2 and 72 ± 2 hours after dose administration. The animals were euthanized at the termination of the study.

JUSTIFICATION FOR SELECTION OF THE TEST SYSTEM

The albino rabbit is recognized as a standard model to predict dermal irritation reaction in humans, and is recommended by the FDA for dermal irritation studies. For this reason, the albino rabbit was used in this study to evaluate the potential dermal irritation reaction caused by the test article.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC)

The care and use of animals on this study was approved by SRC IACUC prior to the initiation of such procedures. IACUC Protocol Approval Date: 25-Aug-2014

IDENTIFICATION OF TEST SYSTEM

Species/Strain:	*Oryctolagus cuniculus*/Albino New Zealand White Rabbits
Source:	Covance
Age:	4.9 months
Weight Range:	2.072 – 2.253 kg
Gender:	Male
Identification:	Ear tag and cage card
No. of Animals Used:	3
In-Life Termination:	Study day 3

HUSBANDRY

Acclimation:	Acclimated at least 5 days prior to dose administration.
Housing:	Animals were single-housed upon receipt.
Environment:	Room temperature and lighting were monitored and controlled while humidity was monitored, but not controlled. All data was maintained as facility records.
Food/Water:	Animals were provided *ad libitum* food and water daily. Food and water analyses are maintained as facility records and there were no known contaminants expected to interfere with the test results.

SELECTION OF ANIMALS

Animals were selected for study based on a physical examination.

ANIMAL PREPARATION

The hair was clipped from the back of each animal prior to dose administration.

TEST ARTICLE PREPARATION

The test article was dosed neat, no dilution or extraction was performed. The pH was tested prior to dosing to verify that the test article was within the acceptable range (≥2 and ≤11.5) for dosing.

TEST AND CONTROL ARTICLE ADMINISTRATION

Each animal received two separate doses. The dose consisted of the control article and the test article. Each dose was intracutaneously injected at five sites (~0.2 mL per site) along one side of the vertebral column. The test article was administered on the right side of each animal while the control article was administered on the left side. The injection sites were separated by at least 1.5 cm. The presence of a bleb at each site after dosing provided verification that each site was successfully dosed.

DERMAL AND GENERAL OBSERVATIONS

All animals were observed daily for general appearance. Dermal observations of the injection sites were performed according to **Table 1** at 24 ± 2, 48 ± 2 and 72 ± 2 hours after dose.

Table 1: Dermal Observation Grading System

Erythema & Eschar Formation	**Score**
No Erythema	0
Very Slight Erythema (barely perceptible)	1
Well-Defined Erythema	2
Moderate to Severe Erythema	3
Severe Erythema (beet red) to Slight Eschar Formation (injuries in depth)	4
Edema Formation	**Score**
No Edema	0
Very Slight Edema (barely perceptible)	1
Slight Edema (edges of area well defined)	2
Moderate Edema (raised approximately 1 millimeter)	3
Severe Edema (raised more than 1 mm and extending beyond the area of exposure)	4
Total possible score for irritation	**8**

Note: Other adverse changes at the injection sites were recorded and reported

TERMINATION

All animals were euthanized with Fatal Plus IV injection at the termination of the study.

EVALUATION CRITERIA

After the 72 ± 2 hours grading, all erythema grades plus edema grades 24 ± 2, 48 ± 2 and 72 ± 2 hours were totaled separately for each test article or control for each individual animal. To calculate the score of the test article or control for each individual animal, divide each of the totals by 15 (3 scoring time points × 5 test or control sample injection sites). To determine the overall mean score for the test article and the control sample, add the scores for the three animals and divide by three. The final test article score was obtained by subtracting the score of the control from the test article score. The requirements of the test were met if the final test article score was 1.0 or less.

ASSAY VALIDITY

The requirements of the test were met as the difference between the test article and control article mean score was 1.0 or less.

METHOD FOR CONTROL OF BIAS

Although the study was not blinded, the use of a control article prevented experimental bias.

ARCHIVAL

The original final report is provided to the Sponsor and a copy of the original report, original protocol (including amendments, deviations, Test Requisition Form and attachments, if applicable) and all original in-life study specific raw data as well as pertinent in-life facility data are archived at Sinclair Research Center LLC.

COMPLIANCE

The procedures including care, housing and handling of animals were performed in compliance with the ISO 10993 Biological Evaluation of Medical Devices – Part 2: Animal Welfare Requirements and facility standard operating procedures. The study was conducted and reported in compliance with the ISO 10993 Biological Evaluation of Medical Devices – Part 10: Tests for Irritation and Skin Sensitization, U.S. Food and Drug Administration Good Laboratory Practice (GLP) regulations set forth in 21 CFR Part 58, and in accordance with the global protocol and the associated Test Requisition Form, facility standard operating procedures, and USDA Policy 12.

POSITIVE CONTROL DATA

Sinclair Research Center will demonstrate positive results using the test system at least every 6 months. A positive control test was completed on 30 November 2015 (SRC study # D10989.050-10). The methods used for this assay were similar to the reported test. The animals used were the same strain. The sensitizer used was 1.5% sodium lauryl sulfate (SLS) in saline and 1.5% SLS in cottonseed oil. The negative control sites were exposed to solvents only.

RESULTS

General Observations

The test and control articles were successfully injected into the intended dosing sites. Daily observations were performed on all animals and all animals appeared normal during the study period.

Dermal Observations and Scoring

Dermal observations are presented in **Tables 2**. Dermal observations from SRC validation study D10989.050-10 are presented in **Tables 3** and **4**.

ANALYSIS AND CONCLUSION

The final score was 0.1 for the test article (device). The final score was based on the dermal observations performed at 24, 48, and 72 hours after dose administration. In the final analysis of data, consideration was given to the overall patterns, intensity, duration, and character of reactions of the test compared with the control conditions. The data indicated that the test article did not cause a skin irritation reaction. In conclusion, the test article is considered a non-irritant under the conditions of this assay.

Table 2: Dermal Observations

Animal ID	Injection Site Score (Erythema/Edema)									
	1		2		3		4		5	
	Ery	Ede	Ery	Ede	Ery	Ede	Ery	Ede	Ery	Ede
Control: 24 h										
0166	1	0	1	0	1	0	1	0	0	0
0167	0	0	1	0	1	0	1	0	1	0
0168	1	0	0	0	1	0	1	0	1	0
Test: 24 h										
0166	0	0	2	0	2	0	2	0	1	0
0167	0	0	0	0	1	0	1	0	1	0
0168	1	0	1	0	1	0	1	0	1	0
Control: 48 h										
0166	0	0	0	0	0	0	0	0	0	0
0167	0	0	0	0	0	0	0	0	0	0
0168	0	0	0	0	0	0	0	0	0	0
Test: 48 h										
0166	0	0	1	0	1	0	0	0	0	0
0167	0	0	0	0	0	0	0	0	0	0
0168	0	0	0	0	0	0	0	0	0	0
Control: 72 h										
0166	0	0	0	0	0	0	0	0	0	0
0167	0	0	0	0	0	0	0	0	0	0
0168	0	0	0	0	0	0	0	0	0	0
Test: 72 h										
0166	0	0	1	0	1	0	0	0	0	0
0167	0	0	0	0	0	0	0	0	0	0
0168	0	0	0	0	1	0	0	0	0	0

Animal ID	Mean Score of Individual Animal	
	Control	Test
0166	0.3	0.7
0167	0.3	0.2
0168	0.3	0.4

Group Mean			Final Score	
	Control	0.3		0.1
	Test	0.4		

Ery= Erythema, Ede= Edema

Table 3: Positive Control Dermal Observations: Saline Formulation

Animal ID	Injection Site Score (Erythema/Edema)									
	1		2		3		4		5	
	Ery	Ede	Ery	Ede	Ery	Ede	Ery	Ede	Ery	Ede
Saline Control: 24 h										
932	0	0	0	0	0	0	0	0	0	0
933	0	0	0	0	0	0	0	0	0	0
934	0	0	0	0	0	0	0	0	0	0
1.5% SLS in Saline: 24 h										
932	4	2	4	3	4	2	4	2	4	3
933	4	3	4	2	4	3	4	2	4	4
934	4	2	4	2	4	2	4	2	4	2
Saline Control: 48 h										
932	0	0	0	0	0	0	0	0	0	0
933	0	0	0	0	0	0	0	0	0	0
934	0	0	0	0	0	0	0	0	0	0
1.5% SLS in Saline: 48 h										
932	4	2	4	3	4	3	4	2	4	4
933	4	3	4	2	4	2	4	2	4	3
934	4	1	4	2	4	2	4	3	4	3
Saline Control: 72 h										
932	0	0	0	0	0	0	0	0	0	0
933	0	0	0	0	0	0	0	0	0	0
934	0	0	0	0	0	0	0	0	0	0
1.5% SLS in Saline: 72 h										
932	4	1	4	2	4	1	4	2	4	3
933	4	3	4	2	4	2	4	1	4	3
934	4	2	4	1	4	1	4	2	4	2

Animal ID	Mean Score of Individual Animal	
	NS Control	NS Extract
932	0.0	6.3
933	0.0	6.5
934	0.0	5.9

Group Mean			Final Score	
	Control	0.0		6.2
	Extract	6.2		

Ery= Erythema, Ede= Edema

Table 4: Positive Control Dermal Observations: Cottonseed Oil Formulation

Animal ID	Injection Site Score (Erythema/Edema)									
	1		2		3		4		5	
	Ery	Ede	Ery	Ede	Ery	Ede	Ery	Ede	Ery	Ede
Cottonseed Oil Control: 24 h										
932	1	0	1	0	1	0	1	0	1	0
933	1	0	1	0	1	0	0	0	1	0
934	0	0	0	0	1	0	1	0	1	0
1.5% SLS in Cottonseed Oil: 24 h										
932	3	1	3	2	3	2	4	2	4	3
933	4	4	4	4	4	3	3	4	3	4
934	3	4	3	4	4	4	4	3	4	3
Cottonseed Oil Control: 48 h										
932	1	0	1	0	1	0	1	0	1	0
933	1	0	1	0	1	0	1	0	1	0
934	0	0	0	0	1	0	1	0	1	0
1.5% SLS in Cottonseed Oil: 48 h										
932	4	3	3	3	3	2	4	3	4	4
933	4	3	4	3	4	2	4	2	4	3
934	4	2	4	3	4	4	4	2	4	3
Cottonseed Oil Control: 72 h										
932	0	0	1	0	1	0	1	0	1	0
933	0	0	1	0	1	0	1	0	0	0
934	0	0	0	0	1	0	1	0	0	0
1.5% SLS in Cottonseed Oil: 72 h										
932	4	2	4	2	3	2	4	1	4	3
933	4	3	4	3	4	2	4	2	4	3
934	4	3	4	1	4	1	4	1	4	2

Animal ID	Mean Score of Individual Animal	
	CSO Control	CSO Extract
932	0.9	5.9
933	0.8	6.9
934	0.5	6.5

Group Mean			Final Score	
	Control	0.7		
	Extract	6.4		5.7

Ery= Erythema, Ede= Edema

REFERENCES

SRC SOP	Sinclair Research Center, LLC, Standard Operating Procedure Manual
ISO 10993-10:2010	Biological Evaluation of Medical Devices Part 10: Tests for Irritation and Skin Sensitization
ISO 10993-12: 2012	Biological Evaluation of Medical Devices: Part 12 – Sample Preparation and Reference Materials
ISO 10993-2:2006/(R)2010	Biological Evaluation of Medical Devices Part 2: Animal Welfare Requirements
FDA 21 CFR-Part 58	Good Laboratory Practice For Nonclinical Laboratory Studies
USDA Policy 12	Consideration of Alternatives to Painful/Distressful Procedures
USP 88	Biological Reactivity Tests IN VIVO