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SPONSOR 14680

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218C0447G-X01G

STUDY NUMBER

18C0447G-X01G

REPORT DATE

May 04, 2018

FINAL REPORT

STUDY TITLE

Maximization Test For Delayed-Type Hypersensitivity in Hartley Guinea Pigs
(ISO 10993-10:2010)

TEST ARTICLE

MRIaudio Ear-Tips
Lot Number: 300-2
Part Number: 350

STUDY DIRECTOR

Zuzana Karjala, Ph.D., RLATg
Senior Scientist
Toxicology

PERFORMING LABORATORY

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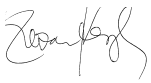
SIGNATURE PAGE

This report is being submitted by the following personnel:

Study Director: Zuzana Karjala, Ph.D., RLATg

05/04/18

X 

I approve the content of this document.
Signed by: Zuzana Karjala

RESPONSIBLE PERSONNEL

1. Roger O'Meara, B.S., LATg, Manager, Toxicology
2. F. Michael Yakes, Ph.D., Executive Vice President
3. Tom Spalding, President

:hc

STATEMENT OF COMPLIANCE

All aspects of the study contained in this report were conducted according to Pacific BioLabs Standard Operating Procedures and in compliance with the United States Food and Drug Administration (FDA) Good Laboratory Practice (GLP) for Nonclinical Laboratory Studies, Title 21 of the U.S. Code of Federal Regulations, Part 58 with the following exception(s):

The facility management was not able to assure the test article was appropriately tested for stability.

Study Director Signature

05/04/18

X 

I approve the content of this document.
Signed by: Zuzana Karjala

QUALITY STATEMENT

QUALITY ASSURANCE UNIT GLP MONITORING AND INSPECTION SUMMARY

In accordance with 21 CFR 58, this study, 18C0447G-X01G, was inspected by Quality Assurance at intervals adequate to assure the integrity of the study. The phase(s) of the study inspected, the date(s) of the inspection, QA auditor, and the date(s) that the QAU inspection report for this study were reported to the Study Director and Management are provided below.

Phase of Study	Date of Inspection	QA Auditor	Date QA Report Provided to Study Director and Management	Date QA Report Acknowledged by Study Director	Date QA Report Acknowledged by Management
Topical Unwrap	04/12/18	KAT	04/12/18	04/13/18	04/13/18

The QAU inspection summary is routinely reviewed by the study director and management of Pacific BioLabs. Management is notified immediately if there are any deviations which might affect the integrity of the study data.

DATA/REPORT REVIEW

Quality Assurance has conducted a thorough review of the test data generated during this study. Report Number 18C0447G-X01G represents an accurate description of the conduct and final results of the study. To the best of my knowledge and ability, this study has been conducted in compliance with applicable Good Laboratory Practice regulations.

Date of Data/ Report Review	Date Review Provided to Study Director and Management	Date Review Acknowledged by Study Director	Date Review Acknowledged by Management
05/03/18	05/03/18	05/03/18	05/03/18

05/04/18

X Kenneth Tan

QA Review
Signed by: Kenneth Tan

STUDY SUMMARY

Purpose: The purpose of this test was to determine to what extent a test article has the potential to act as a contact sensitizer in guinea pigs. This test was conducted according to Pacific BioLabs Standard Operating Procedure (SOP) 16G-41, which follows procedures outlined in ISO 10993-10.

Procedures: The test article was "MRIaudio Ear-Tips." The test article was extracted at 37 ± 1 °C for 72 ± 2 hours in physiological saline and cottonseed oil. Saline and cottonseed oil without the test article were used as negative controls. Undiluted extracts were used for testing.

Thirty-four guinea pigs were used for this study. For each extraction medium, the animals were assigned to two groups; test group (eleven animals) and negative control group (six animals). Positive control animals were tested in a historical positive control study (18B0219G-X01G, completed in April 2018) in order to demonstrate the sensitivity of the assay. In the historical study, 1-chloro-2,4-dinitrobenzene (DNCB) was used as a positive control.

The study was divided into two major phases: the Induction phase and the Challenge phase. During the Induction phase, test animals were exposed intradermally and topically to the test material under evaluation. The negative control animals received control article (saline or cottonseed oil) in the Induction phase.

In the Challenge phase, the test and negative control animals were challenged with the test material. The skin reactions were scored using the Magnusson and Kligman scoring system at 24 and 48 hours after removal of the test article. The skin reactions of the test animals that were exposed to the test article in the Induction phase were compared to the reactions of the unexposed negative control animals. Based on the skin reaction scores observed, the test article was classified as to its allergic potential.

Interpretation: Any skin reaction scores received by the test group, which were greater than the scores received by the negative control group, were considered to represent sensitization. In the final analysis of data, consideration was given to the overall patterns, intensity, duration, and the nature of reactions of the test as compared with the control. No statistical analyses were conducted for the evaluation of data.

Results: On Day 5, one Animal, #35998, (saline test group) was found dead. The death of this animal was not considered to be test article related and the loss of this animal did not affect the integrity of the study since there were a sufficient number of animals left in the group to evaluate test article. All other animals appeared healthy during the course of the study and gained weight at the end of the test. No sensitization reactions or patterns were noted in animals exposed to test article extracted in either saline or cottonseed oil. The test animals did not receive scores higher than those of the negative control animals.

Under similar treatment conditions, all positive control animals used in the historical positive control study exhibited a strong sensitization response to the challenge dose compared to that of the control animals. The results from the historical positive control study demonstrate that the guinea pigs in the study reacted as expected when exposed to a known sensitizer (i.e., DNCB) and the results validate the sensitivity of this test.

Conclusion: The study was performed according to ISO 10993-10 guidelines. According to the criteria for this test, the test article when extracted in saline or cottonseed oil, did not elicit sensitization reactions in animals used in this study.

1. GENERAL INFORMATION

1.1. Study Dates

Study Authorization:	Signed Protocol
Date Test Article Received:	March 26, 2018
Study Initiation Date:	March 28, 2018
Date On Test:	April 04, 2018
Date Off Test:	April 27, 2017
Report Date:	May 04, 2018

1.2. Protocol

This test was conducted according to Protocol Number: 18C0447G-X01G (Appendix II), which incorporates, by reference, SOP 16G-41 and is on file at Pacific BioLabs. There were no amendments to the Protocol.

1.3. Deviations from Protocol

There were no deviations from the Protocol that affected the integrity of the study.

1.4. Key Personnel and Laboratories

Study Director:	Zuzana Karjala, Ph.D., RLATg Senior Scientist, Toxicology Pacific BioLabs 551 Linus Pauling Drive Hercules, CA 94547 United States Phone: (510) 964-9000
Study Sponsor:	Joseph Caruso MRIaudio 2720 Loker Ave W Suite N Carlsbad, CA 92010 United States Phone: (858) 266-8350
Veterinarian:	Erica Weiss-Laroche, DVM Pacific BioLabs 551 Linus Pauling Drive Hercules, CA 94547 United States Phone: (510) 964-9000

2. INTRODUCTION

Purpose: The purpose of this test was to determine to what extent a test article has the potential to act as a contact sensitizer in guinea pigs. This test was conducted according to Pacific BioLabs SOP 16G-41, which follows procedures outlined in ISO 10993-10.

Justification for Test System: Justification for the use of animals in this study is based on the premise that animal testing is an appropriate and ethical prerequisite to testing new medical devices in humans and that data obtained from nonclinical animal models will have relevance to the behavior of the test material in humans. Because of the complex interactions that occur *in vivo*, an *in vitro* system does not provide sufficient information for evaluation of a compound's *in vivo* activities. The use of the guinea pig in this study is specified in current ISO 10993-10 guidelines.

Justification for Number of Animals: The current ISO 10993-10 guidelines require a minimum of ten animals in the test group and a minimum of five animals in the control group be evaluated. In order to assure completion of the study with a sufficient number of animals to meet the ISO 10993-10 guidelines, one additional animal was included in each of the test and control groups.

Justification for Route of Administration: The route of administration was selected based on requirements for the test specified in the ISO 10993-10 guidelines and recommended literature.

Justification for Selection of Extracting Media: The current ISO 10993-12 guidelines require one polar and one non-polar extraction medium be used for this test. Saline (polar) and cottonseed oil (non-polar) extraction media were used as recommended by ISO 10993-12 guidelines.

Justification for Selection of Extraction Conditions: Physicochemical characteristics, material degradation potential, and the final intended use of the test article were considered by the Sponsor when selecting extraction conditions for this study. The extraction conditions (time and temperature) were selected based on recommendations in ISO 10993-12 guidelines.

Dose Rationale: The dose was selected based on recommendations provided by ISO 10993-10 guidelines.

3. MATERIALS AND METHODS

3.1. Test Materials

3.1.1. Test Article Identification

Test Article Name:	MRIaudio Ear-Tips
Physical Description:	Solid
Total Quantity Received for Testing:	1 jar containing ~25 pairs
Quantity Used for This Study:	36 pieces
Lot Number:	300-2
Sample Code:	Not provided by Sponsor
Part Number:	350
Expiration Date:	10/31/2018
Special Handling and/or Precautions:	None
Sterilization Data:	Non-sterile
Storage Conditions:	Room Temperature
Final Intended Use/Application:	Used as an earbud for sound protection and audio

3.1.2. Negative Control Article (1) Identification

Name:	Sterile Saline (0.9% Sodium Chloride Injection, USP)
Manufacturer:	Nova-Tech, Inc.
Physical Description:	Clear liquid
Quantity/Container:	1 Liter/bag
Quantity Used for This Study:	60.0 mL
Lot Number:	A1706041
Expiration Date:	06/2019
Sterility Status:	Sterile
Storage Conditions:	Room Temperature

3.1.3. Negative Control Article (2) Identification

Name:	Cottonseed Oil, NF
Manufacturer:	Spectrum
Physical Description:	Pale yellow, viscous liquid
Quantity/Container:	1 gallon
Quantity Used for This Study:	60.0 mL
Lot Number:	2GH0191
Expiration Date:	07/31/18
Storage Conditions:	2 to 8°C

3.1.4. Test and Control Article Characterization

Test Article: The Sponsor is responsible for all test article characterization specified in the Good Laboratory Practices (GLP) regulations (21 CFR 58.105). Because this is a solid material(s) containing no drug(s), characterization of the test article strength and purity are not considered applicable requirements. The Sponsor has not supplied sufficient information to Pacific BioLabs to assure characterization of the test article meets applicable requirements. Specifically, information that would allow evaluation of the stability of the test article (e.g., shelf life) was not provided. The absence of this information is considered a GLP violation and will be noted in the compliance statement for this report. The Sponsor is responsible for maintaining records of manufacture that would provide information on the composition of the test article and would be able to supply those records if requested by regulatory authorities.

Control Article: The control article was supplied by Pacific BioLabs and information related to the characterization of the control article can be found in Appendix I. The control article was adequately characterized as specified in the Good Laboratory Practices (GLP) regulations (21 CFR 58.105).

3.1.5. Test and Control Article Dose Solution Characterization

Test Article Dose: The test article was extracted by Pacific BioLabs according to ISO 10993-12. The resulting extracts were administered within 24 hours to the test system as specified in ISO 10993-12. Characterization of the extract for strength (concentration), homogeneity, or stability was not conducted. Compliance with the ISO 10993-12 stipulation for use of extracts within 24 hours of preparation is considered adequate to justify the absence of additional characterization.

Control Article Dose: The control article was used without modification. No further characterization of the control article, beyond that provided by the supplier, was conducted.

Text Table 1. Supplies

Item	Lot Number	Manufacturer	Expiration Date
Sterile Saline	A1706041	Nova-Tech, Inc.	06/2019
Cottonseed Oil, NF	2GH0191	Spectrum	07/31/18
Freund's Complete Adjuvant	SLBV6895	Sigma-Aldrich	09/05/19
10% Sodium Dodecyl Sulfate	03/26/18	PBL	04/26/18
Whatman #4 Filter Paper	9719641	GE Healthcare, Whatman	N/A
Surgical Tape (Blenderm)	2022-07AT	3M Health Care	2022-07
Hill Top Chambers®	N/A	Hill Top Research	N/A
Latex Sheeting (Dental Dam)	21518	MSC Industrial Supply Co.	N/A
3-inch Gauze	35383	Dynarex	N/A
Surgical Tape (Zonas)	2947B17	Johnson & Johnson	N/A
Alcohol Swabs (for cleaning sites)	A05231707v	Decon Labs, Inc.	05/19
Alcohol Swabs	023814	Decon Labs, Inc.	01/21

3.1.6. Test and Control Article Description and Preparation

Test Article Description: The test article was "MRIaudio Ear-Tips." Six pieces were used for each extraction.

Test Article Preparation: The test article preparation and extraction conditions are presented in Text Tables 2 and 3. The surface area for one test article was 11.10 cm^2. The total surface area used for each extraction was 66.60 cm^2 and was extracted at a ratio of 60 cm^2/20 mL (wall thickness was greater than 0.05 cm), yielding a volume of 22.2 mL. The test article was made of absorbing materials; therefore, the absorbing capacity was measured in each extraction medium. The absorbed volume was added to the calculated volume.

Test Article Extraction: The extractions were performed according to Pacific BioLabs SOPs. The test article was cut into smaller pieces and immersed in the appropriate extraction medium (saline or cottonseed oil). Prior to extraction, the solutions appeared clear and free of particulates. The test article extraction mixtures were placed in the oven and extracted for 72 ± 2 hours at 37 ± 1°C with agitation.

Control Article Description and Preparation: Physiological saline and cottonseed oil were used as negative controls. Sufficient volumes of control solutions (saline and cottonseed oil without the test article) were prepared in separate glass containers. The control solutions were placed in the oven for 72 ± 2 hours at 37 ± 1°C with agitation.

Text Table 2. Preparation of Test Article and Controls

Total Surface Area (cm^2)	Extraction Ratio (cm^2/mL)	Calculated Volume (mL)	Absorbing Capacity (mL)	Total Volume Test Article (mL)	Total Volume Control (mL)	Extraction		
						Extraction Medium	Temperature (°C)	Duration (hrs)
66.60	60/20	22.2	1.0	23.2	20.0	Saline	37 ± 1	72 ± 2
66.60	60/20	22.2	1.5	23.7	20.0	Cottonseed Oil		

Text Table 3. Test and Control Article Extraction

Phase	Extraction Date (In)	Time (In)	Extraction Date (Out)	Time (Out)
Intradermal Injections	April 01, 2018	0905	April 4, 2018	0830
Topical Application	April 07, 2018	0820	April 10, 2018	0810
Challenge	April 21, 2018	0815	April 24, 2018	0820

Post-Extraction Observations: Following extraction, the extracts were allowed to cool to the touch, shaken well, and decanted into sterile vessels. The test articles and extracts were visually inspected after each extraction. Each test article extract was agitated prior to withdrawal of the injection doses to ensure even distribution of extracted matter. The extracts were kept at room temperature until use.

Saline Extract: The test article extracted in saline was unaffected by the extraction process. After agitation, no particulate matter was noted in the extract. The extract was clear and there were no color changes following the extraction. The extract was used undiluted, unfiltered, and within 24 hours after completion of the extraction process.

Cottonseed Oil Extract: The test article extracted in cottonseed oil was unaffected by the extraction process. After agitation, no particulate matter was noted in the extract. The extract was clear and there were no color changes following the extraction. The extract was used undiluted, unfiltered, and within 24 hours after completion of the extraction process.

3.1.7. Reserve Sample and Sample Disposition

All remaining test articles will be disposed per Pacific BioLabs SOPs or returned to the Sponsor. No reserve samples of the test or control articles will be retained by Pacific BioLabs.

3.2. Test System

Species:	Guinea Pig
Strain:	Hartley, Albino
Source:	Elm Hill Labs, Chelmsford, MA
Number Used:	Thirty-four
Sex:	Male
Age:	Young adult
Initial Weight:	Saline Group: 315 to 389 grams
	Cottonseed Oil Group: 326 to 404 grams
Identification:	Unique identification and cage cards

Environment: Animals were housed either in groups (by gender) or individually in suspended cages. Animals were maintained in a controlled environment at a nominal temperature range of 20 to 26°C, a humidity range of 50 ± 20%, and a light/dark cycle of 12 hours. Animals were maintained in rooms with at least 10 room air changes per hour. Room logs documenting temperature and humidity are kept on file at Pacific BioLabs.

Diet and Feed: Animals received a Certified Laboratory Guinea Pig Diet fortified with vitamin C *ad libitum*. The feed has been analyzed by the supplier for nutritional components and environmental contaminants. There were no known contaminants in the feed that are reasonably expected to interfere with the conduct of this study. All animals received certified food supplement (hay cubes) as a food enrichment during the course of the study.

Water: Fresh, potable drinking water was provided *ad libitum* to all animals via a sipper tube. Water testing is conducted two times a year for total dissolved solids and specified microbiological content and selected elements, heavy metals, organophosphates, and chlorinated hydrocarbons. Results of water analyses are archived at Pacific BioLabs. There are no known contaminants in the water that are reasonably expected to interfere with the conduct of this study. Water was withheld during dosing.

Acclimation: Animals placed on study were acclimated to the testing facility for at least seven days prior to test. Health observations were performed prior to the study to ensure that the animals were acceptable for study use.

Veterinary Care: Veterinary care was available throughout the study as required by changes in clinical signs or other changes. No veterinary treatment was necessary during the course of the study.

Disposition: Disposition of study animals is documented in the Pacific BioLabs study records. Alternate animals not selected for the study were returned to the Pacific BioLabs animal colony for use in subsequent studies or procedures.

3.3. Experimental Design

3.3.1. Assignment to Study Groups

The animal assignments, route of dose administration, and dosing schedule are summarized in Text Table 4. For each extraction medium, the animals were assigned to test and negative control groups. The susceptibility of the guinea pigs to a known sensitizing agent, DNCB, was established in a historical positive control study 18B0219G-X01G, completed in April 2018. The final concentration of DNCB used was 0.050% for the Induction phase and 0.025% for the Challenge phase.

Preliminary Testing: In the case of certain pharmaceuticals (or any test article with potential to cause extensive destruction of the skin), preliminary testing is required to determine the concentration of the test article to be used in the main test. When the test article is an extract of a medical device, it is not likely to cause extensive destruction of the skin. Therefore, this preliminary testing was not necessary and was not performed in this study.

Text Table 4. Study Design Overview

Extracting Medium	Group	Number of Animals (n)	Induction Phase		Challenge Phase
			1st Induction Route of Administration	2nd Induction Route of Administration	Challenge Route of Administration
Saline	Test	11	Intradermal	Topical	Topical
	Negative Control	6	Intradermal	Topical	Topical
Cottonseed Oil	Test	11	Intradermal	Topical	Topical
	Negative Control	6	Intradermal	Topical	Topical

3.3.2. Dosing Procedures

The study consisted of two major phases: the Induction phase and the Challenge phase. During the Induction phase, the animals from the test group were exposed intradermally and topically to the test article extracted in saline or cottonseed oil. The negative control animals received control article (saline or cottonseed oil without the test article). All animals from the test and negative control group were challenged with undiluted test article extract in the Challenge phase.

Induction Phase–Intradermal Injection: The procedure for intradermal injections of the test article extracted in saline and cottonseed oil is presented in Text Table 5 and Figure 1. Prior to dosing, the hair on the dorsocranial thorax of each animal was removed by clipping. The injection sites were disinfected with alcohol wipes.

Three pairs of 0.1 mL intradermal injections were administered to these clipped areas (approximately within a 2 x 4 cm boundary). The first pair of injections (cranial) consisted of an emulsion of Freund's Complete Adjuvant (FCA) in an equal volume of the given vehicle. The second pair of injections (middle) consisted of the test article extract. The third pair (caudal) consisted of an emulsion of the test article extract and an equal volume of FCA.

The negative control animals were treated in a similar manner to the test animals, except that the test article extract was replaced in the second and third pair of injections with an equal amount of control solution (saline or cottonseed oil).

Text Table 5. Induction Phase – Intradermal Injections

Site Location	No. of Sites	Test Group		Negative Control	
		Volume per Site	Dose Preparation	Volume per Site	Dose Preparation
Cranial (A)	2	0.1 mL	1:1 (FCA : SCI/OIL)	0.1 mL	1:1 (FCA : SCI/OIL)
Middle (B)	2	0.1 mL	Test Extract	0.1 mL	Control (SCI/OIL)
Caudal (C)	2	0.1 mL	1:1 (FCA : Test Extract)	0.1 mL	1:1 (FCA : Control SCI/OIL)

FCA – Freund's Complete Adjuvant; SCI – Saline; OIL – Cottonseed Oil

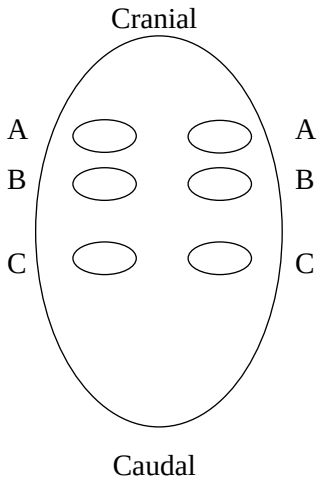


Figure 1: Schematic illustration of dose sites on the dorsum of the animal. A: Equal volumes of FCA and SCI or OIL; B: Test extract or Control; C: Equal volumes of FCA and Test extract or Control

Induction Phase–Topical Application: One day prior to topical application, the previously treated areas were clipped and shaved. The test sites were evaluated for evidence of irritation. Because the test article did not cause irritation, the dosing sites on the test and negative control group animals were pretreated with 10% sodium dodecyl sulfate (SDS) in petrolatum. This SDS mixture was massaged into the skin using a glass rod and left on the site, uncovered, for 24 ± 2 hours.

For the topical application of the Induction phase (7 ± 1 days after the intradermal injections), 4.25 cm diameter disks of Whatman #4 filter paper were each saturated with 0.3 mL of the undiluted test article extract. One of these was applied to the shaved areas on each of the test animals and held in place with surgical tape. The trunks of the animals were wrapped with 3-inch gauze bandage, which was held in place with tape. They were then wrapped with light rubber sheeting (dental dam) so that complete occlusion was obtained. The negative control animals were treated in a similar manner to the test animals except that their patches contained saline or cottonseed oil without the test article. After 48 ± 2 hours, the animals were unwrapped and the patches were removed.

Challenge Phase: The Challenge phase dosing procedure is summarized in Text Table 6. The challenge was performed 14 ± 1 days after the topical application. Prior to challenge, an area on the right side of each animal, measuring approximately 5 x 5 cm, was clipped. On the next day, the right side of each animal was shaved and two Hill Top Chambers® (1 containing 0.3 mL of the test solution and 1 containing 0.3 mL of the control solution) were applied to the shaved areas. All animals (test and control group) were challenged with the undiluted test article extract. The site exposed to saline or cottonseed oil served as a vehicle control.

The animals were wrapped in the same manner as for the topical application of the Induction phase, except that light rubber sheeting was not used because the Hill Top Chambers® provided the necessary occlusion. Twenty-four hours after dosing the animals were unwrapped. The dosing sites were gently cleansed with alcohol wipes to remove any chemical residues.

Text Table 6. Challenge Phase

Extraction Medium	Group	No. of Sites	Volume/Site	Article
Saline	Test	1 1	0.3 mL 0.3 mL	Test Extract Saline
	Negative Control	1 1	0.3 mL 0.3 mL	Test Extract Saline
Cottonseed Oil	Test	1 1	0.3 mL 0.3 mL	Test Extract Cottonseed oil
	Negative Control	1 1	0.3 mL 0.3 mL	Test Extract Cottonseed oil

3.4. In Life Observations and Measurements

3.4.1. Mortality/Moribundity Checks

General morbidity and moribundity checks (cage side observations) were performed once daily.

3.4.2. Clinical Observations

Clinical observations were performed daily. All of the animals were observed for adverse reactions immediately after dosing and daily until the end of the study. Animals were observed for changes in their general appearance including, but not limited to, signs of dehydration, loss of weight, and abnormal posture. Other characteristics observed included appearance of skin and fur, appearance of eyes and mucous membranes, urine and fecal output, and changes in locomotor behavior.

3.4.3. Body Weight Measurement

Body weights were measured prior to the start of the study and at the end of the study.

3.4.4. Scoring

The skin at the challenge dosing sites was scored for skin reaction at 24 ± 2 hours and 48 ± 2 hours after unwrapping according to Magnusson and Kligman scoring criteria (Text Table 7).

Text Table 7. Magnusson and Kligman Scoring System

Patch Test Reaction	Grading Scale
No visible change	0
Discrete or patchy erythema	1
Moderate and confluent erythema	2
Intense erythema and swelling	3

Adopted from ISO 10993–10, *Biological evaluation of medical devices – Tests for irritation and skin sensitization.*

3.5. Interpretation and Analysis

Any skin reaction scores received by the test group, which were greater than the scores received by the negative control group, were considered to represent sensitization. In the final analysis of data, consideration was given to the overall patterns, intensity, duration, and the nature of reactions of the test as compared with the control.

3.6. Statistical Analysis

No statistical analysis was conducted for the evaluation of data.

3.7. Data Acquisition and Analysis

Major computer software systems used on this study included Microsoft Word® and the Rees Scientific Environmental Monitoring System® for study room environmental control.

3.8. Maintenance of Raw Data, Records, and Specimens

Following issuance of the Final Report, records (including, but not limited to, protocol, protocol amendment(s), in-life records, pathology records, dose preparation records, correspondence related to the study, Final Report, and histopathology records) and materials (including, but not limited to, slides, specimens, wet tissues, and blocks) will be archived at Pacific BioLabs (Hercules, CA) for a period of one year. After one year, the Sponsor will be contacted concerning continued storage or return of materials.

Records and materials associated with activities external to Pacific BioLabs (including, but not limited to, clinical pathology, histopathology, and bioanalysis) and activities conducted by the Sponsor (including, but not limited to, dose solution analysis) will be archived by the individual performing laboratories or the Sponsor in a manner consistent with their individual operating SOPs and regulatory requirements.

4. RESULTS AND DISCUSSION

4.1. In Life Observations and Measurements

4.1.1. Survival

On Day 5, Animal #35998 (saline test group) was found dead (See Clinical observations). All other animals survived until scheduled termination. At the end of the study, all animals were euthanized with CO_2 followed by thoracotomy as per Pacific BioLabs SOPs.

4.1.2. Clinical Observations

No test article related abnormalities were noted in any of the tested animals. On Day 5, Animal #35998 (saline test group) was found dead. Gross necropsy was performed. Moderate to severe tissue autolysis present. Severe bloating in the gastrointestinal tract was observed and lungs appeared pale and filled with liquid. The exact cause for death could not be determined at the time of necropsy. There was a sufficient number of animals left in the group to accurately assess the test article but the death of this animal was unlikely test article related since the death occurred early on the study (Day 5) and remaining test group animals were healthy during the course of the study.

All other animals appeared healthy during the course of the study.

4.1.3. Body Weights

The pre-test and post-test body weights are presented in Summary Tables 1 and 2. All of the animals on this study exhibited weight gain over the course of the study.

4.1.4. Scoring

The 24 hour and 48 hour skin reaction scores from the primary challenge are presented in Summary Tables 1 and 2. No sensitization reaction was observed in any of the test animals.

Saline Extraction

Test Group: Three out of ten animals from the test group (Animal #35973, Animal #35984, and Animal #35997) exhibited slight redness (score of 1) on test and control sites on both days of scoring.

Slight redness observed in test animals was not considered to be due to sensitization because similar skin reaction was observed on the control site and in control group. Remaining animals exhibited no skin reaction (score of 0) on either the test or control sites on both days of scoring.

Negative Control Group: Two out of six animals from the control group (Animal #35990 and Animal #35991) exhibited slight redness (score of 1) on test and control sites on both days of scoring. Remaining animals exhibited no skin reaction (score of 0) on either the test or control sites on both days of scoring.

Cottonseed Oil Extractions

Test Group: Three out of eleven animals from the test group (Animal #35978, Animal #35987, and Animal #35996) exhibited slight redness (score of 1) on test and control sites on both days of scoring.

Slight redness observed in test animals was not considered to be due to sensitization because similar skin reaction was observed on the control site and in control group. Remaining animals exhibited no skin reaction (score of 0) on either the test or control sites on both days of scoring.

Negative Control Group: Three out of six animals from the control group (Animal #35981, Animal #35982, and Animal #35993) exhibited slight redness (score of 1) on test and control sites on both days of scoring. Remaining animals exhibited no skin reaction (score of 0) on either the test or control sites on both days of scoring.

Positive Control Group (performed in the historical positive control study): All animals from the test group exhibited erythema ranging from well-defined (score of 2) to intense redness with swelling (score of 3) on the test site and no skin reaction (score of 0) on the control site on both days of scoring. All animals from the negative control group exhibited no skin reaction (score of 0) on either the test or control site on both days of scoring (Summary Table 3). The results from the historical positive control study demonstrate that the guinea pigs reacted as expected when exposed to the sensitizing agent (i.e., DNCB). This validates the sensitivity of the maximization test.

5. CONCLUSION

The study was performed according to ISO 10993-10 guidelines.

Saline Extraction: No sensitization reactions were observed in test animals and the test group animals did not exhibit scores higher than those of the negative control animals.

Cottonseed Oil Extraction: No sensitization reactions were observed in test animals. The test group animals did not exhibit scores higher than those of the negative control animals.

According to the criteria for this test, the test article did not elicit sensitization reactions in the animals used in this study. Under similar treatment conditions, all positive control animals exhibited a strong sensitization response to the challenge dose compared to that of the control. The results from this study demonstrate that the guinea pigs are able to detect sensitizing agent (i.e., DNCB). This validates sensitivity of this test.

6. REFERENCES

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 material*

Pacific BioLabs SOP 16G-41, rev. 6B.00, *Maximization Test for Delayed-Type Hypersensitivity (ISO)*

Maximization Test for Delayed-Type Hypersensitivity – Historical Positive Control, Pacific BioLabs Study Number 18B0219G-X01G, April 2018

Magnusson, B., & Kligman, A. M. (1969). The identification of contact allergens by animal assay. The guinea pig maximization test. *The Journal of Investigative Dermatology*, 52(3), 268-276.

7. SUMMARY OF RESULTS

Summary Table 1. Skin Reaction Scores and Animal Weights (Saline Extraction)

Animal Number	Sex	24 Hour Score		48 Hour Score		Weight (g)	
		Test	Control	Test	Control	Pre-Test	Post-Test
Test Group							
35965	M	0	0	0	0	356	553
35966	M	0	0	0	0	342	533
35967	M	0	0	0	0	315	480
35971	M	0	0	0	0	361	515
35972	M	0	0	0	0	364	571
35973	M	1	1	1	1	342	452
35983	M	0	0	0	0	355	532
35984	M	1	1	1	1	341	502
35985	M	0	0	0	0	352	572
35997	M	1	1	1	1	389	532
35998	M	*-	-	-	-	382	-
Negative Control Group							
35974	M	0	0	0	0	342	512
35975	M	0	0	0	0	365	491
35976	M	0	0	0	0	367	579
35989	M	0	0	0	0	347	512
35990	M	1	1	1	1	382	584
35991	M	1	1	1	1	381	541

*Animal was found dead (see Clinical Observations on Page 17).

Summary Table 2. Skin Reaction Scores and Animal Weights (Cottonseed Oil Extraction)

Animal Number	Sex	24 Hour Score		48 Hour Score		Weight (g)	
		Test	Control	Test	Control	Pre-Test	Post-Test
Test Group							
35968	M	0	0	0	0	394	542
35969	M	0	0	0	0	326	495
35970	M	0	0	0	0	339	507
35977	M	0	0	0	0	380	531
35978	M	1	1	1	1	341	500
35979	M	0	0	0	0	376	553
35986	M	0	0	0	0	362	522
35987	M	1	1	1	1	339	495
35988	M	0	0	0	0	347	495
35995	M	0	0	0	0	347	546
35996	M	1	1	1	1	369	531
Negative Control Group							
35980	M	0	0	0	0	356	521
35981	M	1	1	1	1	383	563
35982	M	1	1	1	1	392	521
35992	M	0	0	0	0	404	585
35993	M	1	1	1	1	351	566
35994	M	0	0	0	0	369	571

Summary Table 3. Skin Reaction Scores and Animal Weights
(Data from Historical Positive Control Study)

Animal Number	Sex	24 Hour Score		48 Hour Score		Weight (g)	
		Test	Control	Test	Control	Pre-Test	Post-Test
Test Group							
35370	M	2	0	2	0	490	658
35371	M	3	0	3	0	432	548
35372	M	2	0	2	0	491	661
35373	M	3	0	3	0	430	534
35375	M	3	0	3	0	353	552
35377	M	3	0	3	0	352	522
Negative Control Group							
35367	M	0	0	0	0	480	631
35368	M	0	0	0	0	410	552
35369	M	0	0	0	0	445	563
35374	M	0	0	0	0	364	519
35376	M	0	0	0	0	385	546
35378	M	0	0	0	0	391	604

APPENDIX I

Certificates of Analysis for Control Articles

NOVA-TECH
PURE PERFORMANCE

CERTIFICATE OF ANALYSIS

PRODUCT:	Sterile Saline
CAT. #:	501032
LOT #:	A1706041
MANUFACTURE DATE:	June 6, 2017
EXPIRATION DATE:	JUN 2019

TEST	SPECIFICATION	RESULT
Sterility	Sterile	Sterile
Volume	≥ 1000 mL	> 1000 mL
Appearance	Clear, colorless	Pass
pH	4.5 - 7.0	5.7
Sodium Chloride Assay	855 - 945 mg/dL	900 mg/dL
Chloride Identification	Pass	Pass
Sodium Identification (Method I)	Pass	Pass
Sodium Identification (Method II)	Pass	Pass
Heavy Metals	≤ 0.001% w/v	Pass
Iron Test	≤ 2 ppm	Pass
Bacterial Endotoxin	≤ 0.5 EU/mL	< 0.1 EU/mL
Particulates ≥ 10 µm	≤ 25 counts/mL	Pass
Particulates ≥ 25 µm	≤ 3 counts/mL	Pass

This product meets specifications and is eligible for release.

21/JUN17

Laboratory Control
21-Jun-17

spectrum® CHEMICAL MFG CORP

Certificate Of Analysis

Item Number	CO145	Lot Number	2GH0191
Item	Cottonseed Oil, NF		
CAS Number	8001-29-4		
Molecular Formula		Molecular Weight	

Test	Specification		Result
	min	max	
ACID VALUE		0.2	0.055
PEROXIDE VALUE		10.0	0.3
UNSAPONIFIABLE MATTER		1.5%	0.37 %
WATER DETERMINATION		0.1%	0.017 %
HEAVY METALS		0.001 %	<10 ppm
ALKALINE IMPURITIES	TO PASS TEST		PASSES TEST
IDENTIFICATION	TO PASS TEST		PASSES TEST
EXPIRATION DATE			31-JUL-2018
DATE OF MANUFACTURE			21-JUL-2017
APPEARANCE			PALE YELLOW LIQUID
RESIDUAL SOLVENTS	TO PASS TEST		PASSES TEST
CLASS 2 (solvent) / HEXANE			<290 ppm

Spectrum Chemical Mfg Corp
755 Jersey Avenue
New Brunswick 08901 NJ

Certificate Of Analysis Results Certified by

Ibad Tirmizi
Director of Quality
Spectrum Chemical Mfg. Corp.

All pharmaceutical ingredients are tested using current edition of applicable pharmacopeia.

Read and understand label and MSDS/SDS before handling any chemicals. All Spectrum's chemicals are for manufacturing, processing, repacking or research purposes by experienced personal only. The customer must ensure to provide its users adequate hazardous material training and appropriate protective gears before handling our chemicals.

APPENDIX II

Protocol

STUDY SPONSOR

MRIaudio
2720 Loker Ave W
Suite N
Carlsbad, CA 92010
United States

GLP Protocol

Maximization Test for Delayed-Type Hypersensitivity in Hartley Guinea Pigs (ISO 10993-10:2010)

Study Number

18C0447G-X01G

PERFORMING LABORATORY

Pacific BioLabs
551 Linus Pauling Drive
Hercules, CA 94547
United States

1. GENERAL INFORMATION

This GLP Protocol (Protocol) describes testing for test and control articles (TACA) submitted by the Sponsor in compliance with the Food and Drug Administration's Good Laboratory Practice (GLP) Regulations (21CFR Part 58). Pacific BioLabs will require a *Laboratory Service Request* (LSR) form with each TACA that details the characteristics of the TACA submitted for testing.

1.1. Study Number

18C0447G-X01G

1.2. Study Title

Maximization Test for Delayed-Type Hypersensitivity in Hartley Guinea Pigs (ISO 10993-10:2010)

1.3. Test Facility

Pacific BioLabs
551 Linus Pauling Dr.
Hercules, CA 94547
United States

1.4. Responsible Personnel

Sponsor's Representative:
Joseph Caruso
MRIaudio
2720 Loker Ave W
Suite N
Carlsbad, CA 92010
United States
Phone: 858-266-8350
E-mail: joe@mriaudio.com

Study Director:
Zuzana Karjala, Ph.D.
Pacific BioLabs
551 Linus Pauling Dr.
Hercules, CA 94547
United States
Phone: 510-964-9000
Email: zuzanakarjala@pacificbiolabs.com

1.5. Proposed Study Dates

The study dates may change due to unexpected events and major delays in the study conduct will be communicated with the Sponsor. The actual study dates will be specified in the Study Report and will not be added by amendment to the Protocol.

Proposed Start Date:	To be determined
Proposed Termination Date:	To be determined
Proposed Report Date:	To be determined

1.6. Alterations to the Protocol

Alterations to the general scope of the Protocol may be made over the period that the Protocol is in effect. Alterations to the Protocol that apply to all subsequent testing will be documented by an amendment to the Protocol and signed and dated by Pacific BioLabs and the Sponsor. In the event that a protocol change is verbally authorized by the Sponsor, Pacific BioLabs will honor the change. However, written

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authorization from the Sponsor will be obtained thereafter. Administrative protocol changes may not require Sponsor signature. All Protocol amendments will be issued to the Sponsor and will be included in the Study Report.

All deviations to the Protocol during the course of a study will be justified by the Study Director as to impact on the study. The deviations that may impact the integrity of the study will be documented in the Study Report.

1.7. Statement of Compliance

These nonclinical laboratory studies will be conducted in accordance with the appropriate Standard Operating Procedures of Pacific BioLabs (Hercules, CA) and the Food and Drug Administration Good Laboratory Practice (GLP) Regulations For Nonclinical Laboratory Studies (21 CFR Part 58). These nonclinical studies will be inspected by the Quality Assurance Unit (QAU) at Pacific BioLabs at intervals adequate to assure the integrity of the studies. QAU inspection findings will be reviewed by the management of Pacific BioLabs; and the Study Director and management will be notified immediately if there are any deviations which might affect the integrity of the study data.

Supporting Studies Conducted by Pacific BioLabs Designated Laboratories. There are no supporting studies conducted by outside laboratories designated by Pacific BioLabs that contribute to this Protocol.

Supporting Studies Conducted by Sponsor. This Protocol does not incorporate supporting studies conducted by the Sponsor. All studies conducted by the Sponsor in conjunction with this Protocol will be reported separately by the Sponsor and will be the sole responsibility of the Sponsor.

1.8. Animal Welfare

In vivo tests will comply with all applicable sections of the Final Rules of the Animal Welfare Act regulations (9 CFR 1-3), the Public Health Service Policy on Humane Care and Use of Laboratory Animals, and the Guide for the Care and Use of Laboratory Animals. Test procedures were reviewed and approved by Pacific BioLabs Institutional Animal Care and Use Committee (IACUC) in compliance with Animal Welfare Act.

Requirement for this study by regulatory agencies is based on the premise that animal testing is a prerequisite for testing new drugs and medical devices in humans, and that animal testing results will predict effects in humans. Because of the complex and multiple interactions that occur *in vivo*, an *in vitro* system would not necessarily provide sufficient information for evaluation of test article toxicity (NIH, 1993). By signature of this protocol, the Sponsor provides assurance that the study is not an unnecessary duplication of previous work, and that documentation for the necessity of this study may be obtained from the Sponsor.

1.9. Safety to the Laboratory

The Sponsor will provide safety information to Pacific BioLabs in the form of a Material Safety Data Sheet (MSDS) for each test article, if available. In the absence of specific safety requirements, standard laboratory safety procedures will be employed for handling the test and control articles, including the use of appropriate personal protective equipment.

1.10. Declaration of Intent

The design and scope of this study are consistent with the overall development strategy of the Sponsor, and this study may be submitted to regulatory agencies, including the United States Food and Drug Administration (FDA).

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2. PURPOSE

The purpose of this test is to determine to what extent a test article has the potential to act as a contact sensitizer in guinea pigs. The test will be performed according to Pacific BioLabs SOP 16G–41, which follows procedures outlined in ISO 10993-10.

2.1. Justification of Test System

Justification for the use of animals in this study is based on the premise that animal testing is an appropriate and ethical prerequisite to testing new medical devices in humans, and that data obtained from nonclinical animal models will have relevance to the behavior of the test material in humans. Because of the complex interactions that occur *in vivo*, an *in vitro* system does not provide sufficient information for evaluation of a compound's *in vivo* activities. The use of the guinea pig in this study is specified in current ISO 10993-10 guidelines.

2.2. Justification of Route of Administration

The intracutaneous and topical applications were selected because they are standard routes of administration for sensitization studies and required by ISO 10993-10 standards.

2.3. Justification for Number of Animals

The current ISO 10993-10 guidelines require a minimum of ten animals in the test group and a minimum of five animals in the control group be evaluated. In order to assure completion of the study with a sufficient number of animals to meet the ISO 10993-10 guidelines, one additional animal is included in each of the test and control groups.

A re-challenge testing may be performed as necessary to resolve any ambiguous or equivocal results. For each re-challenge, five naïve animals will be added to the study (control group) as recommended by ISO 10993-10 guidelines.

2.4. Dose Rationale

Doses are selected based on ISO 10993-10 guidelines and recommendations to maximize exposures.

3. PROCEDURES

3.1. Test Materials

3.1.1. Test and Control Articles

Identification and characterization of test articles will be specified in Study Report of test results, and will not be added by amendment to the Protocol. The following information, supplied by the Sponsor, may be included in the Study Report:

Test Article Name:	MRIaudio Ear-Tips
Physical Description:	Solid
Lot Number:	300-2
Sample Code:	Not provided by Sponsor
Part Number:	350
Expiration Date:	10/31/2018
Special Handling and/or Precautions:	None
Sterilization Data:	Non-sterile
Storage Conditions:	Room Temperature
Final Intended Use:	Used as an earbud for sound protection and audio

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Control Articles will be provided by Pacific BioLabs and will be specified in the Final Report.

Negative Control Article (1) Name:	0.9% Sodium Chloride Injection, USP (SCI)
Physical Description:	Clear liquid
Manufacturer:	Will be provided in the Final Report
Lot Number:	Will be provided in the Final Report
Sterility Status:	Sterile (Passed Parametric Release)
Expiration Date:	Will be provided in the Final Report
Special Handling and/or Precautions:	None
Storage Conditions:	Room Temperature

Negative Control Article (2) Name:	Cottonseed Oil, NF
Physical Description:	Pale yellow, viscous liquid
Manufacturer:	Spectrum
Lot Number:	Will be provided in the Final Report
Sterility Status:	Non-sterile
Expiration Date:	Will be provided in the Final Report
Special Handling and/or Precautions:	None
Storage Conditions:	2 – 8°C

Test and Control Article Characterization. The Sponsor will supply Certificates of Analyses and stability certifications for GLP required characterization of the purity, composition, stability and other pertinent information for the test and control article(s). Similar information for materials (e.g., excipients) used in preparation of dose solutions, if applicable, will be obtained by Pacific BioLabs. Documentation of the characterization of test articles, control articles and excipients (as applicable) will be included in the individual Study Reports. The absence of documentation of the identity, composition, strength and stability of the test articles or control articles (e.g., a CofA) will be considered noncompliance with GLP expectations and will be documented in the Final Report.

The Sponsor's signature and approval of this Protocol indicates that appropriate documentation of the method of synthesis, fabrication or derivation of the test and control article(s) is available to the appropriate regulatory agencies if requested.

Dose Formulation Analysis. Dose formulation analysis will not be conducted.

Reserve Sample and Sample Disposition. Unless requested otherwise, unused test articles or control articles will be discarded or destroyed at the end of the study according to Pacific BioLabs SOPs.

FDA and US Environmental Protection Agency (EPA) regulations require that, for studies of more than four weeks duration, reserve sample from each batch of material be retained for the period of time provided in FDA GLP Regulations 21 CFR Parts 58.105 and 58.195; EPA FIFRA GLP Regulations 40 CFR Parts 160.105 and 160.195; and EPA TSCA GLP Regulations 40 CFR Parts 792.105 and 792.195. The various agencies have, in the past, recommended that the amount of reserve sample be enough to repeat the study two or three times. Sponsor is responsible for retention of test and control article reserves.

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3.2. Test System

Species	Guinea Pig
Strain	Hartley, Albino
Source	Approved Vendor
Number	At least 34
Sex	Male or Female
Age	Young adult
Initial Weight	300 to 500 grams
Identification	Unique Identification and cage card

Environment. Animals will be housed either in groups (by gender) or individually in suspended cages. Animals will be maintained in a controlled environment at a nominal temperature range of 20 to 26°C, a humidity range of 50 ± 20%, and a light/dark cycle of 12 hours. Animals will be maintained in rooms with at least ten room air changes per hour. Room logs documenting temperature and humidity are kept on file at Pacific BioLabs.

Diet and Feed. Animals will receive a Certified Laboratory Guinea Pig Diet fortified with vitamin C *ad libitum*. The feed is analyzed by the supplier for nutritional components and environmental contaminants. There are no known contaminants in the feed that are reasonably expected to interfere with the conduct of this study. It may be necessary during the course of the study to offer supplemental food as part of standard veterinary care. This may not be a certified diet, but will be commercially available food that contains no known contaminants that would interfere with the conduct of this study.

Water. Fresh, potable drinking water will be provided *ad libitum* to all animals via a sipper tube. Water testing is conducted two times a year for total dissolved solids and specified microbiological content and selected elements, heavy metals, organophosphates, and chlorinated hydrocarbons. There are no known contaminants in the water that are reasonably expected to interfere with the conduct of this study. Water will be withheld during dosing.

Acclimation. Animals placed on study will be acclimated to the testing facility for at least 7 days prior to initiation of the study. Health observations will be performed prior to the study to ensure that the animals are acceptable for study use.

Veterinary Care. Veterinary care will be available throughout the study in response to changes in clinical signs or other changes in animal health/welfare. Animals found in severe distress will be immediately treated under the guidance of the attending veterinarian to alleviate pain and suffering. Additional responses to alleviate pain and distress may include a change in dosing paradigm (time, dose, etc.) or a cessation of treatment altogether. Such treatments will be noted in the study files and the Final Reports, and the Sponsor will be notified of the provided veterinary care.

Further, moribund animals may be euthanized at the discretion of the attending veterinarian. Attempts to consult with the study sponsor will occur prior to euthanasia; however, the attending veterinarian will have full authority regarding euthanasia that will be independent of the study Sponsor. Animals removed from the study may be replaced if replacement does not adversely affect the study's conduct and validity, the replacement criteria are recorded and reported, and replacement is done in conformity with relevant Good Laboratory Practices.

Assignment to Study and Disposition. Animals will be examined prior to study initiation, and determined (based on clinical observations) if suitable as test subjects. Eligibility for inclusion on test will be established by the Study Director (or alternate). Disposition of study animals is documented in the Pacific BioLabs study records. Alternate animals not selected for the study will be returned to the Pacific BioLabs animal colony for use in subsequent studies or procedures.

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3.3. Experimental Design

Animal Selection. Only healthy guinea pigs will be used in this study. If females are used, they will be nulliparous and non-pregnant. The initial weight of all animals will be between 300 and 500 grams.

Test Article Preparation. The test article will be extracted according to ISO 10993-12 guidelines and Pacific BioLabs internal SOPs. The test article will be extracted in saline (SCI) and cottonseed oil (OIL) at 37 ± 1°C for 72 ± 2 hours. Extraction volume will be calculated based on the surface area of the test article as specified in ISO10993-12 guidelines. Sufficient volumes of saline and cottonseed oil alone, extracted under the same conditions as test article extracts, will be used as negative controls. Extracts will be stored at room temperature and used within 24 hours after the completion of the extraction process.

Procedure. The study design is presented in Table 1. A total of 34 guinea pigs will be used in this study. The animals will be assigned to four groups; eleven SCI Test animals, six SCI Negative Control animals, eleven OIL Test animals, and six OIL control. The study will be divided into two major phases; the induction phase and the challenge phase. During the induction phase, test animals will be exposed to the test material extracted in appropriate extracting medium (Saline or Cottonseed oil). The test article extract will be administered intradermally and topically. Control animals will be exposed to control solutions.

For the intradermal exposure, animals will receive 3 pairs of 0.1 mL of intradermal injections. One pair will consist of 50:50 (volume ratio) of Freund's Complete Adjuvant mixed with solvent (SCI or OIL). A second pair will consist of undiluted extract. Control animals will be injected with solvent alone. The third pair will consist of the test article extract emulsified with Freund's Complete Adjuvant (50:50 volume ratio). Control animals will receive an emulsion of control solution and Freund's Complete Adjuvant.

Table 1: Study Design

Medium	Group	Number of Animals (n)	Induction Phase		Challenge (14 ± 1 after Topical application)
			Intradermal injection (Day 0)	Topical Application (Day 6-8)	
Saline (SCI)	Test	11	0.1 mL x 2 FCA/SCI	~0.3 mL of Undiluted Test Article Extract (SCI)	~0.3 mL of Undiluted Test Article Extract (SCI)
			0.1 mL x 2 Undiluted Test Extract		
			0.1 mL x 2 FCA/Test Extract		~0.3 mL of SCI Control
	Control	6	0.1 mL x 2 FCA/SCI	~0.3 mL of SCI Control	~0.3 mL of Undiluted Test Article Extract (SCI)
			0.1 mL x 2 Control		
			0.1 mL x 2 FCA/SCI		~0.3 mL of SCI Control
Cottonseed oil (OIL)	Test	11	0.1 mL x 2 FCA/OIL	~0.3 mL of Undiluted Test Article Extract (OIL)	~0.3 mL of Undiluted Test Article Extract (OIL)
			0.1 mL x 2 Undiluted Test Extract		
			0.1 mL x 2 FCA/Test Extract		~0.3 mL of OIL Control
	Control	6	0.1 mL x 2 FCA/OIL	~0.3 mL of OIL Control	~0.3 mL of Undiluted Test Article Extract (OIL)
			0.1 mL x 2 Control		
			0.1 mL x 2 (FCA/OIL)		~0.3 mL of OIL Control

FCA-Freund's Complete Adjuvant; SCI- Saline; OIL- Cottonseed Oil

A topical exposure will be conducted 7 ± 1 day after the intradermal exposure. Twenty four hours ± 2 hours before topical application, intradermal injection sites will be pretreated with 10% sodium dodecyl sulfate (SDS). For the topical application, a filter paper will be saturated with the test extract (~0.3 mL) and applied to the site previously treated with intradermal injections. Similarly, the control animals will receive control solutions (~0.3 mL). The filter paper will be held in place with surgical tape (e.g., Micropore tape). The trunks of the animals will be wrapped with gauze bandage, which will be held in place with porous tape (e.g., Zona's tape). Elastic bandage (e.g., dental dam) will provide a necessary occlusion. The dressing will be removed after 48 ± 2 hours.

Fourteen ± 1 day after the completion of the topical induction phase, all animals will receive two occlusive patches (Hill Top Chambers™). One patch will be saturated with the test article extract

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(~ 0.3 mL) and the other patch with the vehicle control (~ 0.3 mL). The skin at the challenge dosing sites will be scored for skin reaction at 24 ± 2 hours and 48 ± 2 hours after unwrapping according to the criteria provided by the ISO 10993-10 (Table 2). Based on the skin reaction scores observed, the test article will be classified as to its allergic potential. Re-challenge testing may be performed as necessary to resolve any ambiguous or equivocal results.

Historical positive control data will confirm the sensitivity of the assay and the data will be provided in the Final report. A solution of 1-chloro-2,4 dinitrobenzene (0.050% for induction phase and 0.025% for challenge) will be used as a positive control.

Clinical Observation. All of the animals will be observed for adverse reactions immediately after dosing and daily until the end of the study. Animal weights will be recorded at the beginning and the end of the study.

Interpretation and Analysis. Magnusson and Kligman grading scale will be used for grading as recommended by the ISO guidelines (Table 2). Generally, any skin reaction scores received by the test group, which are greater than the scores received by the negative control group, are considered to represent significant sensitization.

In the final analysis of data, consideration will be given to the overall patterns, intensity, duration, and the nature of reactions of the test as compared with the control. No statistical analysis will be conducted for the evaluation of data.

Table 2: Magnusson and Kligman Scale

Grading Scale	Skin Reaction
0	No visible change
1	Discrete or patchy erythema
2	Moderate and confluent erythema
3	Intense erythema and/or swelling

Adopted from ISO 10993-10:2010 Biological Evaluation of Medical Devices – Part 10: Test for Irritation and Skin Sensitization

3.4. In-Life Observations

Mortality/Moribundity Checks. General health of animals will be observed prior and during the test. All of the animals will be observed for adverse reactions immediately after dosing and daily until the end of the study. Abnormal behavior or evidence of poor health will be noted in the study file and the Study Report.

Body Weight. Animal weights will be recorded at the beginning and the end of the study.

3.5. Terminal Procedures and Measurements

Moribund Animals. Animals that show signs of poor health during the course of the study will be evaluated by a veterinarian or designee for possible follow-up treatment.

Post mortem Examinations. Animals that die during the study will be subject to examination or a gross necropsy. Animals sacrificed at the end of study will not be subject to examination or gross pathology. However, females will be checked for signs of pregnancy. At the end of the study, animals will be euthanized as per pacific BioLabs SOPs and disposition of study animals will be documented in the Pacific BioLabs study records. The method of euthanasia will be consistent with the recommendations of the American Veterinary Medical Association guidelines on euthanasia.

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4. DATA ACQUISITION AND ANALYSIS

4.1. Descriptive Statistics

No descriptive statistics will be generated by Pacific BioLabs for these studies.

4.2. Statistical Analysis

No statistical analyses will be performed by Pacific BioLabs for these studies.

5. REPORTS

5.1. General Description of Study Report

The Study Report will include all information necessary to provide a complete and accurate description of the experimental procedures and results. The Study Report will include a compliance statement signed by the Study Director that the report accurately reflects the raw data obtained during the performance of the study and that all applicable GLP regulations were followed in the conduct of the study.

5.2. Study Report

The Study Report will include, but not be limited to, the following:

Name and address of the test facility
Study dates
Study summary
The objective of the study
Test and control article identification
A full description of the test system
A full description of the experimental design and methods
Study results in prose and tabular form as appropriate
Any deviations from the Protocol
Signed statement of compliance from the Study Director

The Study Report will not include results of analyses performed by the Sponsor. Communication of the results of these Sponsor-conducted analyses to the appropriate regulatory agencies will be the responsibility of the Sponsor. Upon finalization, copies of the Final Report will be provided to the Sponsor as hardcopies or PDF files.

6. MAINTENANCE OF RAW DATA, RECORDS AND SPECIMENS

Original data, specimens and reports from this study are the property of the Sponsor. These materials will be available to the Sponsor to facilitate reviewing the study during its progress and before issuance of the Final Report. Records (including, but not limited to, protocol, protocol amendments(s), in-life records, pathology records, dose preparation records, correspondence related to the study, Final Report, and histopathology records) and materials (including, but not limited to, slides, specimens, wet tissues and blocks) will be archived at Pacific BioLabs (Hercules, CA) for a period of one year after issuance of the Final Report. After one year, the Sponsor will be contacted concerning continued storage or return of materials.

Records and materials associated with activities external to Pacific BioLabs (including, but not limited to, clinical pathology, histopathology, and bioanalysis) and activities conducted by the Sponsor (including, but not limited to, dose solution analysis), will be archived by the individual performing laboratories or the Sponsor in a manner consistent with their individual operating SOPs and regulatory requirements.

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7. REFERENCES

ISO 10993-10:2010, *Biological Evaluation of Medical Devices – Tests for Irritation and Skin Sensitization*
ISO 10993-12:2012, *Biological Evaluation of Medical Devices – Sample Preparation and Reference Materials.*
PBL SOP 16G-41, rev. 6B.00, *Maximization Test for Delayed-Type Hypersensitivity (ISO)*
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B. Magnusson, and A.M. Kligman (1969). The identification of contact allergens by animal assay. The guinea pig maximization test. Journal of Investigative Dermatology 52: 268-276.
Good Laboratory Practice Regulations; Food and Drug Administration: 21 CFR Part 58.
Good Laboratory Practice Regulations; Environmental Protection Agency: 40 CFR Part 160
National Institutes of Health. Position statement on the Use of Animals in Research, NIH Guide 22(8), Feb 26, 1993

8. APPROVALS

FOR SPONSOR

Joseph Caruso
Study Sponsor

03/27/18
Date

FOR PACIFIC BIOLABS

Zuzana Karjala, Ph.D.
Pacific BioLabs
Study Director

March 28, 2018
Date

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