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217J0417H-X02G

STUDY NUMBER

17J0417H-X02G

REPORT DATE

November 27, 2017

FINAL REPORT

STUDY TITLE

Intracutaneous (Intradermal) Reactivity Test in New Zealand White Rabbits
(ISO 10993-10:2010)

TEST ARTICLE

MRIaudio Ear-Tips
Lot Number: 300-1
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, Senior Scientist

11/27/17

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

:af

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

11/27/17

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, 17J0417H-X02G, 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
72-hr Observation/ Weighing	11/10/17	ADA	11/13/17	11/13/17	11/13/17

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 17J0417H-X02G 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
11/20/17	11/20/17	11/20/17	11/20/17

11/27/17

X

QA Review
Signed by: Sarah Lauder

STUDY SUMMARY

Purpose: The purpose of this test was to evaluate local responses to extracts of the test article following intracutaneous injections into rabbits. This test was performed according to Pacific BioLabs SOP 16G-43, which follows procedures outlined in ISO 10993-10.

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

Three New Zealand White rabbits were used for this study. Three positive control animals were tested in the historical positive control study (Study Number 17I0323G-X01G, completed in October 2017). In the historical study, Freund's Complete Adjuvant mixed with cottonseed oil was used as a positive control.

The fur on the back of each rabbit was clipped on both sides of the spinal column prior to dosing. A volume of 0.2 mL of the test article extract in saline was injected intracutaneously at five sites on one side of the spinal column, anterior to the midline, of each of three rabbits. A volume of 0.2 mL of the corresponding control was injected intracutaneously at five sites on the other side of the spinal column of the same three rabbits. This process was repeated on the same animal for the cottonseed oil extracts but posterior to the dorsal midline.

The injection sites were observed for erythema, eschar formation, edema, and necrosis, and scored at 24 ± 2 hours, 48 ± 2 hours, and 72 ± 2 hours. The average scores for the test sites were calculated and compared to the average scores for the control sites.

Interpretation: According to ISO 10993-10, the requirements of the test are met if the difference between the test article extract average score and the control average score is 1.0 or less and the test does not fail at any observation period.

Results:

Saline Extract: All animals injected with test article extracted in saline appeared healthy during the course of the study. The overall mean score for the test was 0. The overall mean score for the control was 0. The difference between the overall mean scores was 0. The differences between average test scores and average control scores were not greater than 1.0 at all observation periods.

Cottonseed Oil Extract: All animals injected with test article extracted in cottonseed oil appeared healthy during the course of the study. All animals exhibited slight erythema (score of 1) at test and control sites at 24, 48, and 72 hours. This slight reaction is typically observed in animals injected with cottonseed oil and was not considered to be test article related. No edema was noted at any of the test or control sites during the course of the study. The overall mean score for the test article was 1.0. The overall mean score for the control was 1.0. The difference between the overall mean scores was 0. The differences between average test scores and average control scores were not greater than 1.0 at all observation periods.

Under similar treatment conditions, all positive control animals used in the historical positive control study exhibited a strong irritation response to the positive control and the difference between the positive control average score and the control average was greater than 1.0. In addition, the differences between the positive control and control average scores were greater than 1.0 at all observation periods.

The results from the historical positive control study demonstrate that the rabbits in the study reacted as expected when exposed to a known irritant and the results provide a valid historical positive control for use in this study.

Conclusion: This study was conducted according to ISO 10993-10 guidelines. Based on erythema and edema scores, the test article extracted in saline or cottonseed oil did not elicit biologically significant irritation reactions when compared to the control after being injected intracutaneously. The test article extracted in saline or cottonseed oil met the requirements for the Intracutaneous (Intradermal) Reactivity Test using conditions specified in this report. The results from the historical positive control study demonstrate that the rabbits are able to detect irritating agents. The skin reactions elicited by the positive control validated the sensitivity of this test.

1. GENERAL INFORMATION

1.1. Study Dates

Study Authorization:	Signed Protocol
Date Test Article Received:	October 20, 2017, October 25, 2017
Study Initiation Date:	October 31, 2017
Date On Test:	November 07, 2017
Date Off Test:	November 10, 2017
Report Date:	November 27, 2017

1.2. Protocol

This test was conducted according to Protocol Number: 17J0417H-X02G (Appendix II), which incorporates by reference Standard Operating Procedure 16G-43 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 evaluate local responses to extracts of the test article following intracutaneous injections into rabbits. This test was performed according to Pacific BioLabs SOP 16G-43, 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 rabbit in this study is specified in current ISO 10993-10 guidelines.

Justification for Number of Animals: The New Zealand White rabbit is the species required by ISO 10993-10 guidelines for the Intracutaneous Reactivity Test. The current ISO 10993-10 guidelines require the use of a minimum of three rabbits for each test. The number of animals used in this study was the minimum number that could be used for evaluating the test article.

Justification of Route of Administration: The intracutaneous route of administration was selected because it is required by ISO 10993-10 guidelines.

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:	2 jars each containing ~50 pairs
Quantity Used for This Study:	12 pieces
Lot Number:	300-1
Sample Code:	Not provided by Sponsor
Part Number:	350
Expiration Date:	10/01/2018
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

3.1.2. Negative Control Article (1) Identification

Name:	0.9% Sodium Chloride Injection, USP
Manufacturer:	B. Braun
Physical Description:	Clear liquid
Quantity/Container:	1 Liter/bag
Total Quantity Used for This Study:	20.0 mL
Lot Number:	J7D113
Expiration Date:	04/2019
Sterility Status:	Sterile (Passed Parametric Release)
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
Total Quantity Used for This Study:	20.0 mL
Lot Number:	2GB0321
Expiration Date:	01/31/18
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
0.9% Sodium Chloride Injection, USP	J7D113	B. Braun	04/2019
Cottonseed Oil, NF	2GB0321	Spectrum	01/31/18

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 total 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

Extraction Date (In)	Time (In)	Extraction Date (Out)	Time (Out)
November 04, 2017	0918	November 07, 2017	0805

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: After completion of extraction process, the test article extracted in saline expanded and about one device was no longer immersed but pieces were saturated with extraction medium. The saturated liquid was squeezed out and added to the remaining extract. 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 medium. 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:	Rabbit
Strain:	New Zealand White
Source:	Charles River Laboratories, Wilmington, MA
Number Used:	Three
Sex:	Female (Naïve)
Initial Weight:	2.2 to 2.3 kg
Age:	Adult
Identification:	Ear tags and cage cards

Environment: Animals were housed individually in stainless steel cages. Animals were maintained in a controlled environment at a nominal temperature range of 16 to 22°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 Rabbit Diet (approximately 165 grams per day). The feed was 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.

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 the dosing period.

Acclimation: Animals placed on study were acclimated to the testing facility for at least six days prior to the 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 and was supplied when required by changes in clinical signs or other changes. No veterinary medical treatments were administered during 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 Pacific BioLabs animal colony for use in subsequent studies or procedures.

3.3. Experimental Design

The study design is presented in Text Table 4. Three animals were used in this study. Two extracts were tested in the same set of animals and there were five test and five control sites on each animal for each extract.

Text Table 4. Study Design

Group/Extraction Medium	Number of Animals (n)	Route of Administration	Dose/Site	Number of Sites/Animal	
				Test	Control
Saline	3	Intracutaneous	0.2 mL	5	5
Cottonseed Oil		Intracutaneous	0.2 mL	5	5

3.3.1. Animal Preparation

Prior to test, the fur on the back of each animal was clipped with electric clippers. Only animals with healthy, intact skin were used in this study.

3.3.2. Dosing Procedure

Each extract was vigorously agitated prior to withdrawal of injection doses to ensure even distribution of extracted matter. A volume of 0.2 mL of the test article extracted in saline was injected intracutaneously at five sites on one side of the spinal column, anterior to the midline, of each of three rabbits. A volume of 0.2 mL of saline control was injected intracutaneously at five sites on the other side of the spinal column of the same three rabbits (Figure 1). This process was repeated on the same animals for the test article extracted in cottonseed oil and cottonseed oil control but posterior to the dorsal midline. The dose sites were marked with a permanent marker in order to aid in the identification of dose site locations.

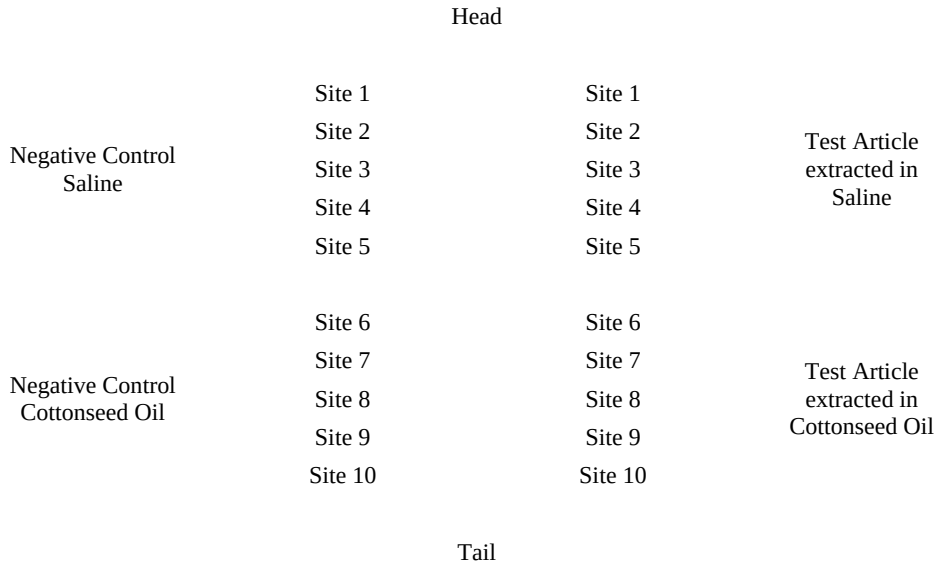

Figure 1: Arrangement of Injection Sites

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 once daily. Animals were observed for changes in their general appearance including, but not limited to, signs of dehydration, loss of weight, abnormal posture, and hypothermia. Other characteristics 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 dosing and at the end of the study.

3.4.4. Scoring

The injection sites were examined immediately after injection, and scored for any tissue reactions, such as erythema, eschar formation, and edema at 24 ± 2 hours, 48 ± 2 hours, and 72 ± 2 hours according to Text Table 5.

Text Table 5. Grading System for Intracutaneous (Intradermal) Reactions

Erythema and Eschar Formation	Score
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate erythema	3
Severe erythema (beet-redness) to eschar formation preventing grading of erythema	4
Edema Formation	Score
No edema	0
Very slight edema (barely perceptible)	1
Well-defined edema (edges of area well defined by definite raising)	2
Moderate edema (raised approximately 1 mm)	3
Severe edema (raised more than 1 mm and extending beyond exposure area)	4
Maximal Possible Score for Irritation	8

Table adopted from ISO 10993-10:2010(E). *Biological evaluation of medical devices – Part 10: Test for irritation and skin sensitization.*

3.5. Interpretation and Analysis

Analysis: After the 72 hour grading, all erythema grades plus edema grades (24, 48, and 72 hrs) were totaled separately for the test sites and control sites for each individual animal. For each individual animal, each of the totals was divided by 15 (3 scoring time points x 5 test and control injection sites). The overall mean scores for each test and corresponding control were calculated by adding the scores for all three animals and dividing by three (total number of animals). The final test score was obtained by subtracting the overall mean score of the control from the overall mean score of the test.

Interpretation: According to ISO 10993-10, the requirements of the test are met if the difference between the test mean score and the control mean score is 1.0 or less and the test does not fail at any observation period. Differences of less than 0 are reported as 0.

If the difference of average reactions is greater than 1.0 at any of the observation periods, the test would fail for that observation period. According to ISO 10993-10, the test would be repeated using three additional rabbits.

3.6. Statistical Analysis

No statistical analyses were performed.

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 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, and histopathology) and activities conducted by the Sponsor 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

No mortality occurred during the study; all animals survived until scheduled termination. At the end of the study, all animals were returned to Pacific BioLabs animal colony as per Pacific BioLabs SOPs.

4.1.2. Clinical Observations

All animals appeared healthy during the course of the study. No test article related clinical signs were observed during the course of the study.

4.1.3. Body Weights

Body weights are presented in Text Table 6. All animals had acceptable body weight when placed on study. All animals exhibited typical body weight at the end of the study.

Text Table 6. Body Weights

Animal Number	Initial Body Weight (kg)	Final Body Weight (kg)	Body Weight Change* (kg)
69371	2.3	2.4	+0.1
69372	2.2	2.4	+0.2
69373	2.2	2.4	+0.2

*Initial body weight was subtracted from final body weight.

4.1.4. Scoring

Scoring: The individual irritation scores are presented in Summary Tables 1 and 2.

Saline Extract: No erythema or edema was observed at any of the test or control sites. The overall mean score for test sites was 0. The overall mean score for control sites was 0. The difference between the overall mean scores (test and control) was 0.

Cottonseed Oil Extract: All animals exhibited slight redness (score of 1) at test and control sites at 24, 48, and 72 hours. Slight redness is typically observed in animals injected with cottonseed oil. Therefore, this slight skin reaction was not considered test article related since a similar reaction was observed at control sites and in control animals. No edema (score of 0) was observed at any of the test or control sites. The overall mean score for test sites was 1.0. The overall mean score for control sites was 1.0. The difference between the overall mean scores (test and control) was 0.

Averages at Each Observation Period: The average reaction scores at each observation period for both saline and cottonseed oil are presented in Summary Table 4. The differences between Average Test Scores and Average Control Scores were less than 1.0 at all observation periods (24, 48, and 72 hours).

Positive Control: The susceptibility of the rabbits to a known irritating agent (Freund's Complete Adjuvant in cottonseed oil) was established in a historical positive control study, study number 17I0323G-X01G, completed in October 2017 (Summary Table 3). The overall mean score for the positive control was 7.0. The overall mean score for the control was 0.8. The difference between the overall mean scores was 6.2. The differences between average positive control scores and average scores were greater than 1.0 at all observation periods (Summary Table 4). The results from this study demonstrated that the rabbits are able to detect irritating agents.

5. CONCLUSION

This study was conducted according to ISO 10993-10 guidelines. All animals appeared healthy during the course of the study. Based on erythema and edema scores, no irritation was observed on test sites when compared to the control. The test article extracted in saline or cottonseed oil met the requirements for the Intracutaneous (Intradermal) Reactivity Test using conditions specified in this report.

Under similar treatment conditions, all positive control animals used in the historical positive control study exhibited strong irritation responses to the positive control. The results from the historical positive control study demonstrate that the rabbits are able to detect irritating agents. The skin reactions elicited by the positive control validate the 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 materials*

Historical Positive Control Study, Study Number 17I0323G-X01G, October 2017

SOP 16G-43, rev. 5A.00, *Intracutaneous (Intradermal) Reactivity Test (ISO)*

7. SUMMARY OF RESULTS

Summary Table 1. Reaction Scores (Saline Extract)

	Test Sites															Control Sites														
Animal ID: 69371	24 ± 2 hrs					48 ± 2 hrs					72 ± 2 hrs					24 ± 2 hrs					48 ± 2 hrs					72 ± 2 hrs				
Erythema	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total reaction score/observation	0					0					0					0					0					0				
Total Mean*	0															0														

	Test Sites															Control Sites														
Animal ID: 69372	24 ± 2 hrs					48 ± 2 hrs					72 ± 2 hrs					24 ± 2 hrs					48 ± 2 hrs					72 ± 2 hrs				
Erythema	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total reaction score/observation	0					0					0					0					0					0				
Total Mean*	0															0														

	Test Sites															Control Sites														
Animal ID: 69373	24 ± 2 hrs					48 ± 2 hrs					72 ± 2 hrs					24 ± 2 hrs					48 ± 2 hrs					72 ± 2 hrs				
Erythema	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total reaction score/observation	0					0					0					0					0					0				
Total Mean*	0															0														

*Total Mean = Total reaction scores/15. Means are rounded to one decimal place.

Interpretation of Results:

Test Overall Mean Score (Total means for all three animals divided by three): 0 / 3 = 0

Control Overall Mean Score (Total means for all animals divided by three): 0 / 3 = 0

Final Test Score (The difference between Test Overall Mean Score and Control Overall Mean Score): 0 – 0 = 0

Summary Table 2. Reaction Scores (Cottonseed Oil Extract)

	Test Sites															Control Sites														
Animal ID: 69371	24 ± 2 hrs					48 ± 2 hrs					72 ± 2 hrs					24 ± 2 hrs					48 ± 2 hrs					72 ± 2 hrs				
Erythema	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Edema	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total reaction score/observation	5					5					5					5					5					5				
Total Mean*	1.0															1.0														

	Test Sites															Control Sites														
Animal ID: 69372	24 ± 2 hrs					48 ± 2 hrs					72 ± 2 hrs					24 ± 2 hrs					48 ± 2 hrs					72 ± 2 hrs				
Erythema	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Edema	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total reaction score/observation	5					5					5					5					5					5				
Total Mean*	1.0															1.0														

	Test Sites															Control Sites														
Animal ID: 69373	24 ± 2 hrs					48 ± 2 hrs					72 ± 2 hrs					24 ± 2 hrs					48 ± 2 hrs					72 ± 2 hrs				
Erythema	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
Edema	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total reaction score/observation	5					5					5					5					5					4				
Total Mean*	1.0															0.9														

*Total Mean = Total reaction scores/15. Means are rounded to one decimal place.

Interpretation of Results:

Test Overall Mean Score (Total means for all animals divided by three): 3.0 / 3 = 1.0

Control Overall Mean Score (Total means for all animals divided by three): 2.9 / 3 = 1.0

Final Test Score (The difference between Test Overall Mean Score and Control Overall Mean Score): 1.0 – 1.0 = 0

Summary Table 3. Reaction Scores (Data from the Historical Positive Control Study)

	Test Sites															Control Sites														
Animal ID: 68937	24 ± 2 hrs					48 ± 2 hrs					72 ± 2 hrs					24 ± 2 hrs					48 ± 2 hrs					72 ± 2 hrs				
Erythema	4	4	4	4	4	3	4	4	4	2	3	3	3	3	3	1	1	1	1	1	1	1	1	1	1	0	1	1	0	0
Edema	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total reaction score/observation	40					37					35					5					5					2				
Total Mean*	7.5															0.8														

	Test Sites															Control Sites														
Animal ID: 68972	24 ± 2 hrs					48 ± 2 hrs					72 ± 2 hrs					24 ± 2 hrs					48 ± 2 hrs					72 ± 2 hrs				
Erythema	3	3	3	3	3	2	2	2	2	3	2	2	2	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0
Edema	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total reaction score/observation	35					31					30					5					5					3				
Total Mean*	6.4															0.9														

	Test Sites															Control Sites														
Animal ID: 68973	24 ± 2 hrs					48 ± 2 hrs					72 ± 2 hrs					24 ± 2 hrs					48 ± 2 hrs					72 ± 2 hrs				
Erythema	4	4	4	3	3	4	4	3	2	2	3	3	3	2	2	1	1	1	1	1	1	1	1	1	1	0	0	1	1	0
Edema	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total reaction score/observation	38					35					33					5					5					2				
Total Mean*	7.1															0.8														

*Total Mean = Total reaction scores/15. Means are rounded to one decimal place.

Interpretation of Results:

Test Overall Mean Score (Total means for all three animals divided by three): 21.0 / 3 = 7.0

Control Overall Mean Score (Total means for all animals divided by three): 2.5 / 3 = 0.8

Final Test Score (The difference between Test Overall Mean Score and Control Overall Mean Score): 7.0 – 0.8 = 6.2

Summary Table 4. Average Reaction Scores at Each Observation Period

Extract	Observation Period	Average Test Score	Average Control Score	Difference
Saline	24 Hr	0	0	0
	48 Hr	0	0	0
	72 Hr	0	0	0
Cottonseed Oil*	24 Hr	1.0	1.0	0
	48 Hr	1.0	1.0	0
	72 Hr	1.0	0.9	0.1
Positive Control (Historical Data)	24 Hr	7.5	1.0	6.5
	48 Hr	6.9	1.0	5.9
	72 Hr	6.5	0.5	6.0

*Intradermal injection of oil frequently elicits some inflammatory response.

APPENDIX I

Certificates of Analysis for Control Articles

B. Braun Medical, Inc.
Certificate of Analysis
11/20/2017

Lot Number: J7D113 Catalog Number: L8000
Product Description: 0.9% Sodium Chloride Injection USP
TIS Number: SPEC-1000051 Issue Date: 6/26/2012
Date of Manufacture: 4/13/2017 Expiration Date: 10/19

Assay	Limits	Result	Units
Total_Chloride_asNaCl	0.855 to 0.908	0.888	%w/v
Dextrose Negative ID	Passes	Passes	
Sodium ID	Passes	Passes	
Chloride ID	Passes	Passes	
pH	4.50 to 7.00	5.08	pH
Iron	Less,Equal to 0.00020	0.00000	%w/v
Heavy_Metals	Less,Equal to 10.00	0.00	ppm
Sterility	Sterile	Sterile	
Bacterial Endotoxins	<= 0.5 EU/mL	Pass	
Particulate Matter	Meets USP Specifications for Particulate Matter for Inj.	Pass	

This is to certify that the above lot was reviewed per manufacturing and Quality Control documents, and complies with all specifications described herein. This lot was manufactured and tested in accordance with its master production documents, USP (as appropriate) and the B. Braun TIS specification referenced above.

Approved By: RDizon Date: 11/21/17
QA/QC Manager or Designee

spectrum® CHEMICAL MFG CORP

Certificate Of Analysis

Item Number	CO145	Lot Number	2GB0321
Item	Cottonseed Oil, NF		
CAS Number	8001-29-4		
Molecular Formula		Molecular Weight	

Test	Specification		Result
	min	max	
ACID VALUE		0.2	0.066
PEROXIDE VALUE		10.0	0.4
UNSAPONIFIABLE MATTER		1.5%	0.43 %
WATER DETERMINATION		0.1%	0.015 %
HEAVY METALS		0.001 %	<10 ppm
ALKALINE IMPURITIES	TO PASS TEST		PASSES TEST
IDENTIFICATION	TO PASS TEST		PASSES TEST
EXPIRATION DATE			31-JAN-2018
DATE OF MANUFACTURE			26-JAN-2017
APPEARANCE			PALE YELLOW OILY 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

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STUDY SPONSOR

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

GLP Protocol

Intracutaneous (Intradermal) Reactivity Test in New Zealand White Rabbits (ISO 10993-10:2010)

Study Number

17J0417H-X02G

PERFORMING LABORATORY

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

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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

17J0417H-X02G

1.2. Study Title

Intracutaneous (Intradermal) Reactivity Test in New Zealand White Rabbits (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

This nonclinical laboratory study 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). This nonclinical study 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

This study 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 PBL's 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 the study is to evaluate local responses to extracts of the test article following intradermal injections into rabbits. This study will be performed according to Pacific BioLabs SOP 16G-43, 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 rabbit in this study is specified in current ISO 10993-10 guidelines.

2.2. Justification of Number of Animals

The current ISO 10993-10 guidelines require a minimum of three animals. The minimum number of animals required for this test will be used.

2.3. Justification of Route of Administration

The intracutaneous route of administration is required by ISO 10993-10 guidelines.

2.4. Dose Rationale

The dose was specified by ISO 10993-10 guidelines.

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:	300-1
Sample Code:	Not provided by Sponsor
Part Number:	350
Expiration Date:	10/01/2018
Special Handling and/or Precautions:	None
Sterilization Data:	Non-sterile
Storage Conditions:	Room Temperature
Final Intended Use:	Used as an earbud for sound protection

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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 Study Report. 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 for prepared test articles.

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	Rabbit
Strain	New Zealand White
Source	Approved Vendor
Number Used	At least three
Initial Weight	At least 2.0 kg
Age	Adult
Gender	Male or Female (Naïve)
Identification	Unique identification and cage cards

Environment. Animals will be housed individually in cages. Animals will be maintained in a controlled environment at a nominal temperature range of 16 to 22°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 Rabbit Diet approximately 165 g per day. 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 6 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

Test Article Preparation. The test article will be prepared according to ISO 10993-12 guidelines and Pacific BioLabs internal SOPs. The test material will be extracted using two extracting media; saline and cottonseed oil. Based on Sponsor's selection, the test article will be extracted at 37 ± 1°C for 72 ± 2 hours. The extraction volume will be calculated based on the surface area of the test article as specified in ISO 10993-12 guidelines. Extracts will be stored at room temperature and used within 24 hours after the completion of the extraction process.

Extracting media (saline and cottonseed oil) without the test article will be used as negative controls. Data from the Historical Positive control study will be provided in the Final report to support the sensitivity of the assay. Freund's Complete Adjuvant in cottonseed oil was used as a positive control.

Procedure. The study design is presented in Table 1. Prior to test, the fur on the back of each animal will be removed. A volume of 0.2 mL of the test article extract obtained with polar (saline) or non-polar (cottonseed oil) extracting medium will be injected at five sites on one side of the back of each rabbit. Similarly, the same volume (0.2 mL) of the polar or non-polar control solution will be injected at five sites on the other side of the back of each rabbit. The injection sites will be observed immediately after injection for erythema, eschar formation, and edema, and scored at 24, 48, and 72 hours according to Table 2.

Table 1: Study Design

Group/Extraction Medium	Number of Animals (n)	Route of Administration	Dose Site	Number of Sites/Animal	
				Test	Control
Saline	3	Intracutaneous	0.2 mL	5	5
Cottonseed Oil		Intracutaneous	0.2 mL	5	5

Table 2: Grading System for Intracutaneous (Intradermal) Reactions

Erythema and Eschar Formation	Score
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate erythema	3
Severe erythema (beet redness) to eschar formation preventing grading of erythema	4
Edema Formation	Score
No edema	0
Very slight edema (barely perceptible)	1
Well-defined edema (edges of area well-defined by definite raising)	2
Moderate edema (raised approximately 1 mm)	3
Severe edema (raised more than 1 mm and extending beyond exposure area)	4
Maximal possible score for irritation:	8

Table adopted from ISO 10993-10:2010(E). Biological evaluation of medical devices – Part10: Test for irritation and skin sensitization. Other adverse changes at the injection sites shall be recorded and reported

Clinical Observation: All of the animals will be observed for adverse reactions immediately after dosing and daily until the end of the study.

Interpretation and Analysis: After the 72 hour grading, all erythema grades plus edema grades (24, 48, and 72 hrs) will be totaled separately for each test sample or control for each individual animal. For each individual animal, each of the totals will be divided by 15 (3 scoring time points x 5 test or control

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injection sites). The overall mean score for test and controls will be calculated by adding the scores for all three animals and divided by three (total number of animals). The final test sample score will be obtained by subtracting the score of the control from the test sample score.

According to ISO 10993–10, if at any observation period the average reaction to the test sample is questionably greater than the average reaction to the control, the test should be repeated using three additional rabbits. The requirements of the test are met if the difference between the test sample and the vehicle blank score is 1.0 or less. Differences of less than 0 will be reported as 0.

3.4. In-Life Observations

Mortality/Morbidity Checks. General health of animals will be observed prior and during the test. If an animal demonstrates an abnormal behavior, shows signs or evidence of poor health the animal will be evaluated by veterinarian or designee. These will be noted in the study file and will be included in the report.

Body Weight. Animals will be weighed prior to dosing and at the end of the study.

3.5. Terminal Procedures and Measurements

Moribund Animals. Animals in severe distress or moribund may be euthanized at the discretion of the consulting veterinarian and the Study Director. The Sponsor will be consulted prior to euthanasia, if possible. Animals removed from the study may be replaced at the discretion of the Study Director, if replacement does not adversely affect study conduct.

Post mortem Examinations. Animals that die during the study will be subjected to a gross necropsy. Animals sacrificed at the end of study will not be subjected to gross necropsy. At the end of the study, animals will be euthanized as per Pacific BioLabs SOPs or returned to Pacific BioLabs animal colony. The 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.

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. REPORT

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.

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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 Reports. Records (including, but not limited to, protocol, protocol amendments(s), in-life records, pathology records, dose preparation records, correspondence related to the study, Final Reports, 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.

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-43, rev. 5A.00, *Intracutaneous (Intradermal) Reactivity Test (ISO)*
PBL ACUP 17A-07, rev. IACUC 6.0, *Intracutaneous Test – Rabbits*
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

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8. APPROVALS

FOR SPONSOR

Study Sponsor

10/30/17
Date

FOR PACIFIC BIOLABS

Zuzana Karjala, Ph.D.
Study Director, Toxicology
Pacific BioLabs

October 31, 2017
Date

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