

# REPORT

**TEST FACILITY**

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CONFIDENTIAL

**STUDY TITLE**

ISO Skin Irritation Study

**TEST ARTICLE NAME**

Amplitude 9oz

**TEST ARTICLE IDENTIFICATION**

Batch: 0455

**NAMSA**

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## Summary

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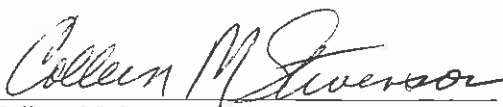
The test article, Amplitude 9oz, Batch: 0455, was evaluated for primary skin irritation in accordance with the guidelines of the International Organization for Standardization 10993. Biological Evaluation of Medical Devices - Part 10: Tests for Irritation and Delayed-Type Hypersensitivity. Two approximate 25 mm x 25 mm sections of the test article and control article were topically applied to the skin of each of three rabbits and left in place for 24 hours. The sites were graded for erythema and edema at 1, 24, 48 and 72 hours after removal of the single sample application.

Under the conditions of this study, very slight erythema and no edema were observed on the skin of the rabbits. The Primary Irritation Index for the test article was calculated to be 0.0. The response of the test article was categorized as negligible.

Study and Supervisory  
Personnel:

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8-25-09  
Date Completed

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## 1. Introduction

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### Purpose

The test article identified below was evaluated for primary skin irritation to determine the potential for a single topical application of the test article to irritate skin of the rabbit.

### Testing Guidelines

The study was conducted based on the International Organization for Standardization 10993, Biological Evaluation of Medical Devices, Part 10: Tests for Irritation and .Delayed-Type Hypersensitivity.

### Dates

Test Article Receipt: August 19, 2009  
Treatment Start Date: August 20, 2009  
Observations Concluded Date: August 24, 2009

## 2. Materials

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The test article provided by the sponsor was identified and handled as follows:

**Test Article Name:** Amplitude 9oz

**Test Article Identification:** Batch: 0455

**Storage Conditions:** Room Temperature

**Control Article:** Four-ply gauze supplied by the test facility, was cut into 25 mm x 25 mm sections, moistened with 0.5 mL of saline per section and backed with polyethylene plastic.

**Control Article Stability Testing:** Marketed product stability characterized by its labeling.

**Control Article Strength, Purity and Composition:** Gauze: Strength: Not applicable, no active components in the formulation; Purity: FDA Quality System Requirements (QSR) as stipulated in 21 CFR Part 820. 20% rayon, 80% polyester blend; Composition: polyester, rayon.

**Preparation:** The test article was cut into approximate 25 mm x 25 mm sections. Either side of each section of the test article was moistened with 0.5 mL of saline and applied to the skin of the rabbit.

## 3. Test System

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### Test System

**Species:** Rabbit (*Oryctolagus cuniculus*)  
**Breed:** New Zealand White  
**Source:** Myrtle's Rabbitry, Inc.  
**Sex:** Male  
**Body Weight Range:** 2.4 kg to 2.5 kg at selection  
**Age:** Young adult  
**Acclimation Period:** Minimum 5 days  
**Number of Animals:** Three  
**Identification Method:** Ear tag

### Justification of Test System

The rabbit is specified as an appropriate animal model for evaluating potential skin irritants by the current ANSI/AAMI/ISO testing standards. The rabbit is widely used for this purpose and relative ranking of irritant scores can be determined.

#### 4. Animal Management

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Husbandry:	Conditions conformed to NAMSA Standard Operating Procedures that are based on the " <i>Guide for the Care and Use of Laboratory Animals.</i> "
Food:	A commercially available rabbit feed, PROLAB Hi-Fiber Rabbit - 5P25, was provided daily.
Water:	Potable water was provided <i>ad libitum</i> through species appropriate water containers or delivered through an automatic watering system.
Contaminants:	Contaminants reasonably expected in feed or water supplies were not believed to have influenced the outcome of this test.
Housing:	Animals were individually housed in stainless steel suspended cages identified by a card indicating the lab number, animal number, test code, sex, and date dosed.
Environment:	The animal housing room temperature and relative humidity were monitored daily. The temperature for the room was 61-72°F and the relative humidity was 30-70%. There were no significant temperature or relative humidity excursions that adversely affected the health of the animals.  The light cycle was controlled using an automatic timer (12 hours light, 12 hours dark).
Accreditation:	NAMSA is an AAALAC International accredited facility and is registered with the United States Department of Agriculture. Additionally, NAMSA maintains an approved Animal Welfare Assurance on file with the National Institutes of Health, Office for Laboratory Animal Welfare.
Personnel:	Associates involved were appropriately qualified and trained.
Selection:	Only healthy, previously unused, animals free from irritation or other dermatological lesions that could interfere with the test were selected.
Veterinary Care:	Standard veterinary medical care was provided in this study.
IACUC:	This procedure has been approved by NAMSA Institutional Animal Care and Use Committees (IACUC), and is reviewed at least annually by the same committees. Any significant changes to this procedure were approved by the IACUC prior to conduct.

#### 5. Method

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The animals were weighed and the fur on the back of each rabbit was clipped with an electric clipper 4 to 24 hours prior to treatment. On the day of treatment, four sites, two on each side of the back and positioned cranially and caudally, were designated on each rabbit. The sites were free of blemishes that could interfere with the interpretation of results.

An approximate 25 mm x 25 mm section of the test article was moistened with 0.5 mL of saline, and applied to one cranial site and one caudal site (two sites per rabbit) by introduction under a 4 ply gauze layer to an area of skin approximately 25 mm x 25 mm square. The patches were backed with plastic and covered with a nonreactive tape. The control sample was similarly applied to the opposite cranial and caudal sites. The trunk of each animal was wrapped with an elastic binder to maintain the test patches in position. Animals were returned to their cages after treatment.

After the 24 hour exposure, the binders, tape, and patches were removed. The sites were gently wiped with a gauze sponge dampened with deionized water in an attempt to remove any remaining residue.

##### Laboratory Observations

1. Animals were observed daily for general health.
2. Body weights were recorded for each animal at pretreatment.
3. Dermal observations for erythema and edema were recorded at 1, 24, 48 and 72 hours after patch removal in accordance with the criteria in Appendix 1.

All times and temperatures reported herein are approximate and are within ranges established by the external standards described in the References section of this report and/or NAMSA standard operating procedures.

## 6. Evaluation and Statistical Analysis

The Primary Irritation Index of the test was calculated following test completion for each animal. The erythema and edema scores obtained at the 24, 48 and 72 hour intervals were added together and divided by the total number of observations. This calculation was conducted separately for the test and control article for each animal. The score for the control was subtracted from the score for the test article to obtain the Primary Irritation Score. The Primary Irritation Score for each rabbit was added together and divided by the number of rabbits to obtain the Primary Irritation Index. The Primary Irritation Index was characterized based on the definitions outlined in Appendix 1.

## 7. Results

All animals appeared clinically normal throughout the study. Individual results of dermal scoring appear in Appendix 2. Very slight erythema and no edema reactions resulted in an overall evaluation of negligible irritation to the skin of the rabbits. The time of onset of the Maximum Irritation Response was at 1 hour and the time to Maximum response was 1 hour. The Primary Irritation Index of the test article was calculated to be 0.0. The irritation calculations are shown below:

Rabbit Number	Test Score Average	-	Control Score Average	Individual Primary Irritation Score	Combined Primary Irritation Score (CPIS)	Primary Irritation Index (CPIS ÷ 3)	Response Category
63981	0.0	-	0.0	0.0	0.0	0.0	Negligible
63982	0.0	-	0.0	0.0			
63983	0.0	-	0.0	0.0			

## 8. Conclusion

Under the conditions of this study, very slight erythema and no edema were observed on the skin of the rabbits. The Primary Irritation Index for the test article was calculated to be 0.0. The response of the test article was categorized as negligible.

Results and conclusions apply only to the test article tested. Any extrapolation of these data to other samples is the sponsor's responsibility. All procedures were conducted in conformance with good manufacturing practices and certified to ISO 13485:2003.

## 9. Records

All raw data pertaining to this study and a copy of the final report are retained in designated NAMSA archive files.

## 10. References

Code of Federal Regulations (CFR), Title 9, Parts 1-199, Animal Welfare Act (2008).

National Research Council, *Guide for the Care and Use of Laboratory Animals*, Washington, DC: National Academy Press, 1996.

Office of Laboratory Animal Welfare (OLAW), Public Health Service Policy on Humane Care and Use of Laboratory Animals.

Code of Federal Regulations (CFR), Title 16, Part 1500, Federal Hazardous Substances Act (FHSA) Regulations (2008).

International Organization for Standardization (ISO) 10993-10, Biological Evaluation of Medical Devices - Part 10: Tests for Irritation and Delayed-Type Hypersensitivity (2002).

International Organization for Standardization (ISO) 10993-2, Biological Evaluation of Medical Devices - Part 2: Animal Welfare Requirements (2006).

**Appendix 1 - Classification System For Skin Reaction**

REACTION	NUMERICAL GRADING
Erythema and Eschar Formation	
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	
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
Total possible score for irritation	8

NOTE: Other adverse changes at the skin sites shall be recorded and reported

IRRITATION RESPONSE CATEGORIES IN THE RABBIT

RESPONSE CATEGORY	MEAN SCORE
Negligible	0.0 to 0.4
Slight	0.5 to 1.9
Moderate	2.0 to 4.9
Severe	5.0 to 8.0

**Appendix 2 - Dermal Observations**

Rabbit Number/ Sex	Weight (kg)	Group	Observation	Interval (hours)							
				1		24		48		72	
				Left	Right	Left	Right	Left	Right	Left	Right
63981 Male	2.5	Test	Erythema	0	0	0	0	0	0	0	0
			Edema	0	0	0	0	0	0	0	0
		Control	Erythema	0	0	0	0	0	0	0	0
			Edema	0	0	0	0	0	0	0	0
63982 Male	2.5	Test	Erythema	0	1	0	0	0	0	0	0
			Edema	0	0	0	0	0	0	0	0
		Control	Erythema	0	0	0	0	0	0	0	0
			Edema	0	0	0	0	0	0	0	0
63983 Male	2.4	Test	Erythema	1	0	0	0	0	0	0	0
			Edema	0	0	0	0	0	0	0	0
		Control	Erythema	0	0	0	0	0	0	0	0
			Edema	0	0	0	0	0	0	0	0