GLP REPORT

TEST FACILITY:

NAMSA
6750 Wales Road
Northwood, OH 43619

SPONSOR:

Paul Tiege
ViRexx Medical Corporation
8223 Roper Road NW
Edmonton, Alberta, T6E 6S4
Canada

CONFIDENTIAL

STUDY TITLE:

ISO Maximization Sensitization Study - Extract

TEST ARTICLE:

Occlusion® 500 Artificial Embolization Device

IDENTIFICATION NO.:

Batch: FL288

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Summary

A guinea pig maximization test of Occlusion® 500 Artificial Embolization Device, Batch: FL288, was conducted to evaluate the potential for delayed dermal contact sensitization. This study was conducted based on the requirements of the International Organization for Standardization 10993: Biological Evaluation of Medical Devices, Part 10: Tests for Irritation and Delayed-Type Hypersensitivity.

The test article was extracted in 0.9% sodium chloride USP (SC) and sesame oil, NF (SO). Each extract was intradermally injected and occlusively patched to ten test guinea pigs (per extract) in an attempt to induce sensitization. The vehicle was similarly injected and occlusively patched to five control guinea pigs (per vehicle). Following a recovery period, the test and control animals received a challenge patch of the appropriate test article extract and the reagent control. All sites were scored at 24 and 48 hours after patch removal.

Under the conditions of this study, the SC and SO test article extracts showed no evidence of causing delayed dermal contact sensitization in the guinea pig.

Study and Supervisory Personnel:

Diane L. Miller
Natasha N. Norris, B.S.
Courtney M. Craft, B.S.
Molly F. Corvo, B.S.
Nicole L. Pence, A.A.
Lindsey E. Roberts, B.S.
Carrie A. Fetter
Ericka N. McCalla, B.S.
Mark J. Menzel, RVT, A.A.S.
Shelli L. Snyder, A.A.
Valerie D. Gnepper, B.S.

Approved by:

Michelle E. Longstreet

Michelle E. Longstreet, B.S.
Study Director

8-23-07
Date Completed

Statement of GLP Compliance

This study was conducted in accordance with the provisions of the FDA Good Laboratory Practice (GLP) Regulations (21 CFR, Part 58).

There were no deviations from the protocol, standard operating procedures or the GLP Regulations which were judged to have had any significant impact on the validity or interpretation of the data.

All laboratory data has been accurately recorded and verified, as indicated by the signature below.

Study Director: Michelle E. Longstreet, B.S. 8-23-07 Date

1. Introduction

Purpose
A guinea pig maximization test of the material identified below was conducted to evaluate the potential to cause delayed dermal contact sensitization. This study was conducted based on the requirements of the International Organization for Standardization 10993: Biological Evaluation of Medical Devices, Part 10: Tests for Irritation and Delayed-Type Hypersensitivity.

Dates
The test article was received on May 30, 2007 and June 27, 2007. Treatment began on July 10, 2007, and the observations were concluded August 5, 2007.

GLP Compliance
The study initiated by protocol signature on June 11, 2007, was conducted in accordance with the provisions of the FDA Good Laboratory Practice (GLP) Regulations, 21 CFR 58. A Statement of Quality Assurance Activities was issued with this report.

Duplication of Experimental Work
By signature on the protocol, the sponsor confirmed that the conduct of this study did not unnecessarily duplicate previous experiments.

2. Materials

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

Test Article:	Occlusion® 500 Artificial Embolization Device
Identification No.:	Batch: FL288
Stability Testing:	In progress (per sponsor)
Expiration Date:	Stable for duration of intended testing (per sponsor)
Strength, Purity and Composition:	The sponsor elected not to provide this information to NAMSA and takes full responsibility for this data and can supply this information if requested to do so.
Physical Description of the Test Article:	Glass vials containing white beads
Storage Conditions:	Refrigerate
Vehicles:	0.9% sodium chloride USP solution (SC) Sesame oil, NF (SO)
Preparation:	A 7.3 ml portion of each extract was added to the original container in order to remove the test article from the original container. The test article was prepared based on the sponsor supplied surface area of 44 cm^2 per sample. Three vials were included in each preparation. A 7.3 ml portion of each extract was added to the original container in order to remove the test article from the original container. Based on a ratio of 120 cm^2:20 ml, a 44.0 cm^2 portion of the test article was covered with 7.3 ml of the vehicle. The test article was extracted with agitation in SC and SO at 37°C for 72 hours. The vehicles (without test article) were similarly prepared to serve as the reagent control.

Condition of Extracts:

	SC Test	SC Control
Induction I:	clear with particulates	clear
Induction II:	clear with particulates	clear
Challenge:	clear	clear

	SO Test	SO Control
Induction I:	clear with particulates	clear
Induction II:	clear with particulates	clear
Challenge:	clear with particulates	clear

Additional Materials: Freund's Complete Adjuvant (FCA) was mixed 50:50 (v/v) with the chosen vehicle and used at induction I. A 10% (w/w) sodium lauryl sulfate (SLS) suspension in petrolatum was used for induction II. These materials were provided by the test facility.

Sample Disposition: Per GLP regulations 21CFR58.105(d), sample from each batch must be maintained for studies longer than 4 weeks duration for the period of time designated in GLP regulations 21CFR58.195. Samples from each batch will be archived at the following location:

NAMSA
6750 Wales Road,
Northwood, OH 43619

3. Test System

Test System

Species:	Guinea pig (*Cavia porcellus*)
Strain:	Hla®:(HA)CVF®
Source:	Hilltop Lab Animals, Inc.
Sex:	Female (nulliparous)
Body Weight Range:	316 grams to 379 grams at study initiation
Age:	Young adult
Acclimation Period:	Minimum 5 days
Number of Animals:	Thirty
Identification Method:	Ear punch

Justification of Test System

The Hartley albino guinea pig has been used historically for sensitization studies (Magnusson and Kligman, 1970). The guinea pig is believed to be the most sensitive animal model for this type of study. The susceptibility of the Hartley guinea pig strain to a known sensitizing agent, 1-chloro-2,4-dinitrobenzene (DNCB), has been substantiated at NAMSA with this method under lab number 06T_58332_02 completed on January 15, 2007.

4. Animal Management

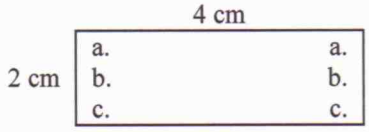
Husbandry:	Conditions conformed to Standard Operating Procedures that are based on the "Guide for the Care and Use of Laboratory Animals."
Food:	A commercially available guinea pig feed was provided daily.
Water:	Potable water was provided *ad libitum* through species appropriate water containers or delivered through an automatic watering system.
Contaminants:	Reasonably expected contaminants in feed or water supplies did not have the potential to influence the outcome of this test.
Housing:	Animals were housed in groups in stainless steel suspended cages identified by a card indicating the lab number, animal numbers, test code, sex, animal code and first treatment date.

Environment:	The room temperature was monitored daily. The temperature range for the room was within a range of 64-79°F. The room humidity was monitored daily. The humidity range for the room was 30-70%. 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 were selected.
Sedation, Analgesia or Anesthesia:	Sedation, analgesia or anesthesia was not necessary during the routine course of this procedure.
Veterinary Care:	In the unlikely event that an animal became injured, ill, or moribund, care was conducted in accordance with current veterinary medical practice. If warranted for humane reasons, euthanasia was conducted in accordance with the current report of the American Veterinary Medical Association's Panel on Euthanasia. The objective of the study will be given due consideration in any decision and the study sponsor will be advised.
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

On the first day of treatment, fifteen guinea pigs per extract (ten test, five control) were weighed and identified. The fur over the dorsoscapular region was removed with an electric clipper.

Induction I
The test animals were injected with the test article extract and the control animals were injected with the reagent control. Three rows of intradermal injections (two per row) were given to each animal within an approximate 2 cm x 4 cm boundary of the fur clipped area as illustrated below:



Control Animals:
a. 0.1 ml of 50:50 (v/v) mixture of FCA and the chosen vehicle
b. 0.1 ml of vehicle
c. 0.1 ml of a 1:1 mixture of the 50:50 (v/v) vehicle/FCA mixture and the vehicle

Test Animals:
a. 0.1 ml of 50:50 (v/v) mixture of FCA and the chosen vehicle
b. 0.1 ml of test extract
c. 0.1 ml of a 1:1 mixture of the 50:50 (v/v) vehicle/FCA mixture and the test extract

To minimize tissue sloughing the "a" and "c" injections were slightly deeper than "b". Site "c" was injected slightly more caudal than site "b".

Induction II
The day prior to conducting the Induction II patch, the fur over the dorsoscapular region (same area as used during induction I) was removed with an electric clipper and the area was treated with 0.5 to 1 gram of a 10% sodium lauryl sulfate (SLS) suspension in petrolatum. The SLS suspension, applied to provoke a mild acute inflammation, was massaged into the skin over the injection site. The area was left uncovered.

At 7 days (±1 day) after completion of the Induction I injection, any remaining SLS residue was gently removed with a gauze pad. A 2 cm x 4 cm section of filter paper, saturated with approximately 0.3 ml of freshly prepared test article extract, was then topically applied to the previously injected sites of the test animals. The control animals were similarly patched with the appropriate reagent control. Each patch was secured with a nonreactive tape and the trunk of each animal was wrapped with an elastic bandage. At 48 hours, the binders and patches were removed.

Challenge

At 14 days (±1 day) after unwrapping the Induction II wraps, the fur was removed from the sides and flank areas with an electric clipper. The nonwoven cotton disk contained in a Hill Top Chamber® was saturated with approximately 0.3 ml of the test article extract or reagent control. The test extract was applied to the right flank of each animal and the control vehicle was applied to the left flank of each animal. Each patch was secured to the skin with semiocclusive hypoallergenic adhesive tape. The trunk of each animal was wrapped with an elastic bandage to maintain well-occluded sites. At 24 hours, the wraps and patches were removed and any residue remaining at the sites was removed.

Laboratory Observations

1. Animals were observed daily for general health.
2. Body weights were recorded at pretreatment.
3. Observations for dermal reactions were conducted at 24 and 48 hours after challenge patch removal. Prior to each scoring interval, the sites were wiped with 35% isopropyl alcohol. If necessary, the fur was clipped from each site to facilitate scoring. Scores were recorded in accordance with the criteria shown below:

Patch test reaction	Grading scale
No visible change	0
Discrete or patchy erythema	1
Moderate and confluent erythema	2
Intense erythema and swelling	3

6. Evaluation and Statistical Analysis

The responses from the challenge phase were compared within the test animal group and between test and control conditions. Control conditions were (1) the vehicle control solution on the test animals and (2) the test extract, control solution and biomaterial (if applied) on the control animals.

In the final analysis of data, consideration was given to the overall pattern, intensity, duration and character of reactions of the test as compared to the control conditions. Statistical manipulation of data was not applicable to this study. Grades of 1 or greater in the test group generally indicated sensitization, provided that grades of less than 1 were observed on the control animals. If grades of 1 or greater were noted on control animals, then the reactions of test animals that exceeded the most severe control reaction were considered to be due to sensitization.

7. Results

Body Weights and Clinical Observations

Individual body weights are presented in Appendix 1. All animals appeared clinically normal throughout the study.

Dermal Observations

Individual results of dermal scoring for the challenge phase appear in Appendix 2. No evidence of sensitization was observed.

8. Conclusion

Under the conditions of this study, the SC and SO test article extracts showed no evidence of causing delayed dermal contact sensitization in the guinea pig.

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, certified to ISO 13485:2003 and accredited to ISO 17025:2005.

9. Quality Assurance

Inspections were conducted at intervals adequate to assure the integrity of the study in conformance with 21 CFR 58.35(b)(3). The final report was reviewed for conformance to Section 58.185, Subpart J, of the GLP Regulations. A Statement of Quality Assurance Activities is provided with this final report.

10. Proposed Dates

The study dates were finalized by the study director following receipt of the sponsor approved protocol and appropriate material for the study. Initiation of the study was the date on which the study director signed the GLP protocol. Projected dates for starting the study (first treatment) and for the completion of the study (final report release) were provided to the sponsor (or representative of the sponsor).

11. Records

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

12. References

21 CFR 58 (GLP Regulations).

Guide for the Care and Use of Laboratory Animals, Institute for Laboratory Animal Research, National Academy of Sciences (Washington: National Academy Press, 1996).

ISO 10993-10 (2002) Biological evaluation of medical devices - Part 10: Tests for irritation and delayed-type hypersensitivity.

Magnusson, B. and A. Kligman, *Allergic Contact Dermatitis in the Guinea Pig* (Springfield: C.H. Thomas, 1970).

OLAW, Public Health Service Policy on Humane Care and Use of Laboratory Animals (NIH Publication)

United States Code of Federal Regulation (CFR) 9: The Animal Welfare Act.

13. Protocol Changes

Any necessary changes to the protocol after sponsor approval or study initiation were documented and approved by the study director as protocol amendments. Copies were distributed to the sponsor, the raw data file, and the NAMSA Quality Assurance department.

Appendix 1 - Individual Body Weights and Clinical Observations

SC Group

Group	Animal Number	Individual Observation	
		Pretreatment Body Weight (g)	Clinical Observations
Test	1	344	Animal appeared clinically normal throughout the study.
	2	333	Animal appeared clinically normal throughout the study.
	3	353	Animal appeared clinically normal throughout the study.
	4	364	Animal appeared clinically normal throughout the study.
	5	321	Animal appeared clinically normal throughout the study.
	6	371	Animal appeared clinically normal throughout the study.
	7	319	Animal appeared clinically normal throughout the study.
	8	320	Animal appeared clinically normal throughout the study.
	9	366	Animal appeared clinically normal throughout the study.
	10	344	Animal appeared clinically normal throughout the study.
Control	11	328	Animal appeared clinically normal throughout the study.
	12	316	Animal appeared clinically normal throughout the study.
	13	349	Animal appeared clinically normal throughout the study.
	14	371	Animal appeared clinically normal throughout the study.
	15	349	Animal appeared clinically normal throughout the study.

Appendix 1 (continued) - Individual Body Weights and Clinical Observations

SO Group

Group	Animal Number	Individual Observation	
		Pretreatment Body Weight (g)	Clinical Observations
Test	16	374	Animal appeared clinically normal throughout the study.
	17	347	Animal appeared clinically normal throughout the study.
	18	350	Animal appeared clinically normal throughout the study.
	19	352	Animal appeared clinically normal throughout the study.
	20	346	Animal appeared clinically normal throughout the study.
	21	379	Animal appeared clinically normal throughout the study.
	22	339	Animal appeared clinically normal throughout the study.
	23	349	Animal appeared clinically normal throughout the study.
	24	340	Animal appeared clinically normal throughout the study.
	25	357	Animal appeared clinically normal throughout the study.
Control	26	354	Animal appeared clinically normal throughout the study.
	27	351	Animal appeared clinically normal throughout the study.
	28	351	Animal appeared clinically normal throughout the study.
	29	333	Animal appeared clinically normal throughout the study.
	30	352	Animal appeared clinically normal throughout the study.

Appendix 2 - Dermal Reactions – Challenge

SC Group

Group	Animal Number	Hours Following Patch Removal			
		24 Hour Score		48 Hour Score	
		Control	Test Extract	Control	Test Extract
Test	1	0	0	0	0
	2	0	0	0	0
	3	0	0	0	0
	4	0	0	0	0
	5	0	0	0	0
	6	0	0	0	0
	7	0	0	0	0
	8	0	0	0	0
	9	0	0	0	0
	10	0	0	0	0
Control	11	0	0	0	0
	12	0	0	0	0
	13	0	0	0	0
	14	0	0	0	0
	15	0	0	0	0

Appendix 2 (continued) - Dermal Reactions – Challenge

SO Group

Group	Animal Number	Hours Following Patch Removal			
		24 Hour Score		48 Hour Score	
		Control	Test Extract	Control	Test Extract
Test	16	0	0	0	0
	17	0	0	0	0
	18	0	0	0	0
	19	0	0	0	0
	20	0	0	0	0
	21	0	0	0	0
	22	0	0	0	0
	23	0	0	0	0
	24	0	0	0	0
	25	0	0	0	0
Control	26	0	0	0	0
	27	0	0	0	0
	28	0	0	0	0
	29	0	0	0	0
	30	0	0	0	0

Statement of Quality Assurance Activities

Phase Inspected	Auditor	Date
SLS Application	K. J. Evener	July 16, 2007
Induction 2	K. J. Evener	July 17, 2007
Final Report Review	K. J. Evener	August 23, 2007

Reports to Management and Study Director(s)	Date
Periodic Status Report Periodic Status Report	July 10, 2007 August 10, 2007

This study will be included in the next periodic status report as completed.

Based on a review of this study, it has been concluded that this report accurately describes the methods and standard operating procedures, and that the reported results accurately reflect the raw data of the study. This study has been reviewed in accordance with the provisions of the FDA Good Laboratory Practice Regulations (21 CFR, Part 58).

QA Representative: Karen J. Evener

Karen J. Evener, B.E.
Auditor, Quality Assurance

Aug. 23, 2007
Date

STORE IN REFRIGERATOR
(+4°C)
CALIBRATION #: 7420
TECH/DATE: VB 5-30-07

GLP SAMPLE S[illegible]ION FORM

USA Corporate Headquarters
6750 Wales Rd
Northwood, Ohio 43619
T 866.666.9455 (toll free)
F 419.662.4386

C[illegible]
F 949.951.[illegible]

Ohio
6750 Wales Rd
Northwood, Ohio 43619
T 866.666.9455
F 419.666.2954

07T_37252
25447_001 25447

-required field

SPONSOR FINAL REPORT WILL BE ADDRESSED AND MAILED TO

ViRexx Medical Corp — Paul Tiege
COMPANY NAME* — **ATTN***
8223 Roper Road
ADDRESS*
Edmonton — Alberta — T6E 6S4
CITY* — **STATE*** — **ZIP***
Canada
COUNTRY*
780 989 6715
PHONE*
780 436 0068
FAX*
ptiege@virexx.com
E-MAIL*

INVOICE INFORMATION
same, Attn. Erin Horwitz
BILLING ADDRESS (include Company Name if different from mailed to)*

V0725-186PT
PURCHASE ORDER NUMBER*
T07 2708
COST ESTIMATE AND PROPOSAL NUMBER
☐ VISA ☐ MasterCard ☐ American Exp.
CARD HOLDER NAME
CREDIT CARD NUMBER — **EXPIRATION DATE**
ACCOUNTS PAYABLE PHONE* — **ACCOUNTS PAYABLE FAX***

Occlusin® 500 Artificial Embolization Device
TEST ARTICLE NAME *USE EXACT WORDING DESIRED ON FINAL REPORT* * +

Embolotherapy
INTENDED CLINICAL USE OF TEST ARTICLE:*

X BATCH ☐ CODE ☐ LOT — FL288
CHECK ONE — **IDENTIFICATION NUMBER***

CONTROL ARTICLE NAME*

☑ BATCH ☐ CODE ☐ LOT
CHECK ONE — **IDENTIFICATION NUMBER***
NAMSA recommends only one lot, batch, or code per test article submission.

QUANTITY SUBMITTED:* 38 vials Occlusin® 500 Artificial Embolization Device , Batch FL288
(please specify quantities for each lot/batch/code provided)
PHYSICAL DESCRIPTION OF TEST ARTICLE (Chemical/Material type/Color)*
glass vials containing white beads

TEST ARTICLE IS CATEGORIZED AS BEING A (check all that apply):* +
MEDICAL DEVICE ☐ BIOLOGIC ☐ TISSUE
☐ PHARMACEUTICAL ☐ CHEMICAL ☐ OTHER

+ A detailed composition list and current MSDS sheet must accompany any chemical or biologic test article. A certificate of testing or reprocessing must be submitted for any human tissue derived sample or clinically used medical device

TEST ARTICLE BEING SUBMITTED IS:*
STERILIZED ☐ NOT STERILIZED
☐ **NAMSA TO STERILIZE BY:** ☐ EO (additional charge) ☐ STEAM

Mixtures of test or control articles with carriers require analysis to demonstrate proper concentration, homogeneity, and stability.*
☐ Sponsor will provide analytical methods; or
☐ Sponsor will perform analysis on representative aliquots provided by NAMSA.

STORAGE CONDITIONS*
☐ ROOM TEMPERATURE **X REFRIGERATION** ☐ FREEZER
☐ OTHER:

TEST AND CONTROL ARTICLE CHARACTERIZATION: The sponsor assures the above test article has been characterized for identity, strength, purity, and composition as required by FDA Good Laboratory Practice Regulations of 21 CFR Part 58.105. Stability testing is the responsibility of the sponsor and is subject to FDA audit. Characterization and stability information are also required for control articles. Please check the statement(s) applicable to the test and control articles for both Stability and Strength, Purity and Composition sections below.

Test Article	Control Article	Stability (Choose One)
X	☐	Stability testing is in progress; article is stable for duration of intended testing.
☐	☐	Stability testing is complete and on file with sponsor. Expiration date (test): Expiration date (control):
☐	☐	Marketed product stability characterized by its labeling.

Test Article	Control Article	Strength, Purity, and Composition (Choose One)
☐	☐	Sponsor provided data in a Certificate of Analysis or other appropriate documentation and results will be reflected in the final report.
X	☐	Sponsor elects not to provide this information to NAMSA and takes full responsibility for this data and can supply this information if requested to do so.

If requesting to return sample, please check the courier and include your:
☐ UPS ☐ Federal Express ☐ Other: _____ Account Number: _____

T053007_017
FEDEX VIREXX 5-30-07 Recirculation Ice Packs

AUTHORIZED BY SPONSOR — DATE 28 MAY 07
NAMSA STUDY DIRECTOR Michelle E. Longstreet — DATE 6-11-07

REV040207

TEST FACILITY:

NAMSA
6750 Wales Road
Northwood, OH 43619-1011

SPONSOR:

Paul Tiege
ViRexx Medical Corporation
8223 Roper Road NW
Edmonton, Alberta,
Canada

STUDY TITLE:

ISO Maximization Sensitization Study - Extract

10993-10

TABLE OF CONTENTS

Approvals

Sponsor Representative (Sponsor): Paul D

Date Approved: 18 MAY 07

Study Director (NAMSA): Michelle E. Longstreth

Date Initiated: 6-11-07

1. Introduction

Purpose
The purpose of this study is to identify the potential for dermal sensitization. The Magnusson and Kligman method has been effective in identifying a variety of allergens. This study will be based on the requirements of the International Organization for Standardization 10993: Biological Evaluation of Medical Devices, Part 10: Tests for Irritation and Delayed-Type Hypersensitivity.

GLP Compliance
Good Laboratory Practice – This nonclinical laboratory study will be conducted in accordance with the United States Food and Drug Administration Good Laboratory Practice Regulations, 21 CFR Part 58.

Duplication of Experimental Work
By signature on this protocol, the sponsor confirms that the conduct of this study does not unnecessarily duplicate previous experiments.

2. Materials

Test Article
The sponsor will submit the test article to be evaluated. Detailed information about the test article will be provided by the sponsor on the NAMSA Sample Submission Form or on a similar attachment to the protocol.

Preparation
The following is to be completed by the sponsor or study director. Further instructions may be attached to the protocol. The sample will be prepared as follows:

(1) **Ratio of test article to extraction vehicle (select one):**

- ✓ Material thickness less than 0.5 mm - ratio of 120 cm^2:20 ml
- ___ Material thickness greater than or equal to 0.5 mm - ratio of 60 cm^2:20 ml
- ___ Irregularly shaped objects and/or sponsor option - ratio of 4 g:20 ml
- ___ Other (explain): _____

(1) **Test Article Preparation Instructions:**

each vial of occlusim 505 has a total SA per vial of 44 cm^2
please extract 3 vials, 132 cm^2, in an appropriate volume for each of SC & vegetable oil extractions

extraction procedure should be done under constant agitation, e.g. end-over-end, to prevent particles from clumping

(1) **Extraction Vehicle (select all that apply):**

- ✓ 0.9% sodium chloride USP solution (SC)
- ✓ ~~Vegetable oil~~ sesame oil MEL 6-1-07
- ___ Other (specify): _____

(1) **Extraction Conditions (select one):**

- ✓ 37°C, 72 hours
- ___ 50°C, 72 hours
- ___ 70°C, 24 hours
- ___ 121°C, 1 hour
- ___ Other (specify): _____

(1) The test article itself is suitable for topical application at the challenge phase.

- ___ Yes
- (No)

(1) completed by sponsor MEL 6-1-07

① **Disposition of Test/Control Article (select one):**

✓ Discard ____ Return unused article ____ Return unused and used article

For studies >28 days in life, NAMSA will retain a representative portion of the test/control article.

Special Laboratory Instructions:

Control Article
The vehicle used to prepare the extract will be prepared in the same manner as the extract (but without test article) to serve as the control measure. Untreated skin will serve as an additional control reference for scoring dermal reactions during the challenge phase.

3. Test System

Test System

Species:	Guinea pig (*Cavia porcellus*)
Strain:	Hartley
Source:	NAMSA approved supplier
Sex:	No particular gender is prescribed for this test. If females are used, they will be nulliparous and not pregnant
Body Weight Range:	300-500 grams at study initiation
Age:	Young adults
Acclimation Period:	Minimum 5 days
Number of Animals:	Minimum of fifteen (per extract)
Identification Method:	Ear punch

Justification of Test System
The Hartley albino guinea pig has been used historically for sensitization studies (Magnusson and Kligman, 1970). The guinea pig is believed to be the most sensitive animal model for this type of study. The susceptibility of the Hartley strain to a known sensitizing agent, 1-chloro-2,4-dinitrobenzene (DNCB) has been substantiated at NAMSA with this method.

4. Animal Management

Husbandry:	Conditions will conform to Standard Operating Procedures that are based on the "Guide for the Care and Use of Laboratory Animals."
Food:	A commercially available guinea pig feed will be provided daily.
Water:	Potable water will be provided *ad libitum* through species appropriate water containers or delivered through an automatic watering system.
Contaminants:	Reasonably expected contaminants in feed or water supplies should not have the potential to influence the outcome of this test.
Housing:	Animals will be housed in groups in stainless steel suspended cages identified by a card indicating the lab number, animal numbers, test code, sex, animal code and first treatment date.
Environmental:	The room temperature will be monitored daily. The recommended temperature range for the room is 64-79°F. The room humidity will be monitored daily. The humidity range for the room is 30-70%. The light cycle will be controlled using an automatic timer (12 hours light, 12 hours dark).

①completed by sponsor MEL 6-1-07

Facility:	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 will be appropriately qualified and trained.
Selection:	Only healthy animals will be selected.
Sedation, Analgesia or Anesthesia:	It has been determined that the use of sedation, analgesia or anesthesia will not be necessary during the routine course of this procedure.
Veterinary Care:	In the unlikely event that an animal should become injured, ill, or moribund, care will be conducted in accordance with current veterinary medical practice. If warranted for humane reasons, euthanasia will be conducted in accordance with the current report of the American Veterinary Medical Association's Panel on Euthanasia. The objective of the study will be given due consideration in any decision and the study sponsor will be advised.
IACUC:	This protocol 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 protocol must be approved by the IACUC prior to conduct.

5. Test and Control Article Preparation

Fresh extracts will be prepared at each phase of the study as previously indicated (see Test Article). If the test material is suitable for patching, a topical application of the test sample (2 cm x 2 cm patch) will be used at the challenge. The vehicle used to prepare the extract will be prepared in the same manner as the extract (but without test article) to serve as the control measure.

6. Method

On the first day of treatment, fifteen guinea pigs per extract (ten test, five control) will be weighed and identified. The fur from the dorsoscapular area of the animals will be removed with an electric clipper.

Induction I
Three pair of intradermal injections will be administered to the animals within an approximate 2 cm x 4 cm area over the dorsoscapular region as follows:

Control Animals
a. 0.1 ml of 50:50 (v/v) mixture of Freund's Complete Adjuvant (FCA) and the chosen vehicle
b. 0.1 ml of vehicle
c. 0.1 ml of a 1:1 mixture of the 50:50 (v/v) FCA and the vehicle

Test Animals
a. 0.1 ml of 50:50 (v/v) mixture of FCA and the chosen vehicle
b. 0.1 ml of test extract
c. 0.1 ml of a 1:1 mixture of the 50:50 (v/v) FCA and the test extract

To minimize tissue sloughing the "a" and "c" injections will be slightly deeper than "b". Site "c" will be injected slightly more caudal than site "b".

Induction II
The day prior to conducting the Induction II patch, the injection sites will be clipped free of fur again and treated with 0.5 to 1 g of a 10% (w/w) sodium lauryl sulfate (SLS) suspension prepared by mixing the powdered SLS with petrolatum unless the animals exhibit excessive redness and/or swelling at site b. At 7 days (±1 day) after completion of the Induction I injection, any remaining SLS residue will be gently wiped from the area with gauze.

A 2 cm x 4 cm filter paper patch, saturated with approximately 0.3 ml of the extract preparation or vehicle, will be applied over the same injection area and secured with a nonreactive tape. The trunk of each animal will then be wrapped snugly with an elastic band for 48 hours (±2 hours).

Challenge
At 14 days (±1 day) after unwrapping induction II wraps, the fur will be clipped from the sides and flanks with an electric clipper. A nonwoven cotton disk backed by a flexible chamber (e.g. Hill Top Chamber®) and semiocclusive hypoallergenic tape, will be saturated with approximately 0.3 ml of freshly prepared test material extract and applied to the right flank or dorsum of each animal. In addition, the vehicle control will be patched to the left flank or dorsum of each animal. An approximate 2 cm x 2 cm section of test material itself (if appropriate) will be applied to the right flank.

The trunk of each animal will be wrapped to maintain well-occluded sites. At 24 hours (±2 hours) the wraps and patches will be removed and any residue remaining at the sites will be wiped with gauze.

Laboratory Observations
1. Animals will be observed daily for general health.
2. Body weights will be recorded at pretreatment.
3. Observations for dermal reactions will be conducted at 24 and 48 hours after patch removal. Prior to each scoring interval, the sites will be wiped with 35% isopropyl alcohol. If necessary, the fur will be clipped from each site to facilitate scoring. Dermal sensitization results will be compared between the test and control animals in accordance with the criteria shown below:

Patch test reaction	Grading scale
No visible change	0
Discrete or patchy erythema	1
Moderate and confluent erythema	2
Intense erythema and swelling	3

Rechallenge
Should the original challenge results prove to be equivocal, the animals may be rechallenged with a fresh test extract and vehicle control approximately 1 – 2 weeks after the first challenge patch application. The rechallenge will be conducted in the same manner as the challenge but at virgin sites on the opposite flank. After the test is completed, all animals will be handled in accordance with IACUC approved NAMSA procedures.

7. Evaluation and Statistical Analysis

In the final analysis of data, consideration will be given to the overall pattern, intensity, duration, and character of reactions of the test as compared to the control conditions. Statistical manipulation of data is not applicable to this study. Grades of 1 or greater in the test group generally indicate sensitization, provided that grades of less than 1 are observed on the control animals. If grades of 1 or greater are noted on control animals, then the reactions of test animals that exceeded the most severe control reaction will be considered to be due to sensitization.

For rechallenge results, the overall pattern, intensity, duration and character of reactions seen will be compared between the challenge and rechallenge. Recurring observations in at least one of the same animals will be considered as verification of earlier findings.

8. Report

A final report will be issued to include a description of the methods, the resulting data in tabular format and conclusions.

9. Quality Assurance

Inspections will be conducted at intervals adequate to assure the integrity of the study in conformance with 21 CFR 58.35(b)(3). The final report will also be reviewed for conformance to Section 58.185, Subpart J, of the GLP Regulations. A Statement of Quality Assurance Activities will be provided with the final report.

10. Proposed Dates

The study dates will be finalized by the study director following receipt of the sponsor-approved protocol and appropriate material for the study. Initiation of the study will be the date on which the study director signs the GLP protocol. Projected dates for starting the study (first treatment) and for the completion of the study (final report release) will be provided to the sponsor (or representative of the sponsor).

11. Records

Test article preparation, animal weights, treatment procedures, dermal reaction scores, and dates of relevant test activities from study initiation to completion will be recorded.

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

12. References

21 CFR 58 (GLP Regulations).

Guide for the Care and Use of Laboratory Animals, Institute for Laboratory Animal Research, National Academy of Sciences (Washington: National Academy Press, 1996).

ISO 10993-10 (2002) Biological evaluation of medical devices - Part 10: Tests for irritation and delayed-type hypersensitivity.

Magnusson, B. and A. Kligman, *Allergic Contact Dermatitis in the Guinea Pig* (Springfield: C.H. Thomas, 1970).

OLAW, Public Health Service Policy on Humane Care and Use of Laboratory Animals (NIH Publication)

United States Code of Federal Regulation (CFR) 9: The Animal Welfare Act.

13. Protocol Changes

Any necessary changes to the protocol after sponsor approval or study initiation will be documented and approved by the study director as protocol amendments. Copies will be distributed to the sponsor, the raw data file, and the NAMSA Quality Assurance department.

NAMSA

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June 12, 2007

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PROTOCOL AMENDMENT I

Test Article: Occlusin® 500 Artificial Embolization Device

Identification: Batch: FL288

NAMSA Submission ID.: 07T_37252

We have received appropriate test article and approved protocol(s) for the program to be conducted in accordance with the Good Laboratory Practice (GLP) Regulations on the material described above. Below is a projected schedule for the work to be performed.

NAMSA Code	NAMSA Lab Number	Study	Estimated Start Date:	Estimated Report Release Date:
TI261_300	07T_37252_02	ISO Maximization Sensitization Study - Extract - 0.9% SC Extract	June 25, 2007	August 24, 2007
TI261_300	07T_37252_03	ISO Maximization Sensitization Study - Extract - SO Extract	June 25, 2007	August 24, 2007
TI251_800	07T_37252_04	ISO Intracutaneous Study - Extract - 0.9% SC Extract	June 18, 2007	July 12, 2007
TI251_800	07T_37252_05	ISO Intracutaneous Study - Extract - SO Extract	June 18, 2007	July 12, 2007
TS200_901	07T_37252_06	Two Week Rat Study, Repeated Parenteral Administration of Two Extracts - 0.9% SC Extract	June 25, 2007	September 21, 2007
TS200_901	07T_37252_07	Two Week Rat Study, Repeated Parenteral Administration of Two Extracts - SO Extract	June 25, 2007	September 21, 2007

Michelle E. Longstreet

Michelle E. Longstreet, B.S.
Study Director

6-12-07
Date

cc: QA (NAMSA)
GLP study file