TEST FACILITY

NAMSA
9 Morgan
Irvine, CA 92618
949.951.3110

SPONSOR

Mike Stewart
IMBiotechnologies LTD
Suite 113 – Advanced 9650 20th Avenue
Edmonton, Alberta T6N 1G1
Canada

STUDY TITLE

USP Pyrogen Study - Material Mediated

TEST ARTICLE NAME

Occlusin 505 Artificial Embolization Device

TEST ARTICLE IDENTIFICATION

FL288

TABLE OF CONTENTS

Page

Summary

The test article, Occlusin 505 Artificial Embolization Device, FL288, was extracted in sterile, nonpyrogenic 0.9% sodium chloride solution. The test solution was evaluated in the rabbit for material mediated pyrogenicity. The test was conducted based on USP General Chapter <151> Pyrogen Test. The procedure is recommended in ISO 10993-11, Biological Evaluation of Medical Devices - Part 11: Tests for Systemic Toxicity.

A single dose of 10 mL/kg was intravenously injected via the marginal ear vein into each of three rabbits. Rectal temperatures were measured and recorded prior to injection and at 30 minute intervals between 1 and 3 hours after injection.

Under the conditions of this study, the total rise of rabbit temperatures during the 3 hour observation period was within acceptable USP limits. The test article was judged as nonpyrogenic.

Study and Supervisory Personnel:

John A. Muraski, Ph.D.
Manager, In Vitro & Toxicology

Approved by:

Marcia Mestre, B.S.
Study Director

10-30-09
Date Completed

pm

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: _____ 10-30-09

Marcia Mestre, B.S. Date

1. Introduction

Purpose
The test article identified below was prepared and evaluated for material mediated pyrogenicity. The purpose of this study was to determine whether an extract of the test article induced a pyrogenic response following intravenous injection in rabbits. *In vivo* biological reactivity was evaluated following a single injection of the extract.

Testing Guidelines
The study was conducted based on the United States Pharmacopeia, National Formulary, General Chapter <151>, Pyrogen Test. The procedure is recommended in International Organization for Standardization 10993-11, Biological Evaluation of Medical Devices - Part 11: Tests for Systemic Toxicity.

Dates
Test Article Receipt: October 16, 2009
Test Conducted Date: October 26, 2009

GLP Compliance
The study initiated by protocol signature on October 16, 2009, 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 the 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.

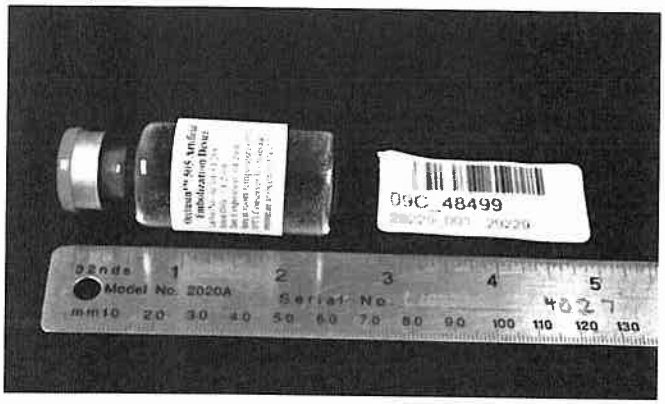
2. Materials

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

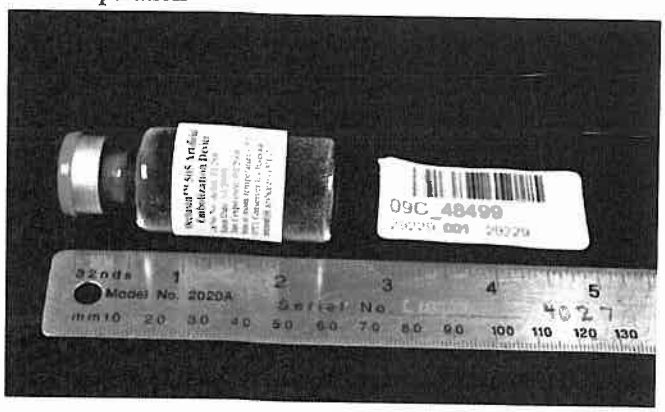
Test Article Name: Occlusin 505 Artificial Embolization Device

Test Article Identification: FL288

Pre-Preparation



Post-Preparation



Stability Testing: In progress (per sponsor)

Expiration Date: Stable for duration of intended testing (per sponsor)

Strength, Purity and Composition: Strength: Not applicable because no active ingredients are used to formulate a concentration; Purity: Not applicable because the test article is a multi-component device; Composition: polyactide – co-glycolide and bovine collagen.

Physical Description of the Test Article: Microspheres supplied as a dry powder in sterile sealed glass vials, 400 mg/vial

Storage Conditions:	Refrigerate	
Sham Solution:	Sterile, nonpyrogenic 0.9% sodium chloride solution (SNPS), warmed to 37°C	
Vehicle:	Sterile, nonpyrogenic 0.9% sodium chloride solution (SNPS)	
SNPS Stability:	Marketed product stability characterized by its labeling	
SNPS Strength, Purity and Composition:	Strength:	Not applicable; no active components in the formulation
	Purity:	Meets requirements of USP Sodium Chloride for Injection and is certified as USP Grade. 0.9% NaCl ± 5.0% of label claim, balance is water
	Composition:	CAS #: 7647-14-5, sodium chloride/water CAS #: 7732-18-5
Preparation:	Based on a ratio of 120 cm^2: 20 mL, a 901.3 cm^2 portion of the test article was covered with 150 mL of SNPS and extracted with continuous agitation at 37°C for 72 hours. The test extract was warmed in a 37°C water bath for a minimum of 10 minutes prior to injection.	
Condition of Extract:	Clear	

3. Test System

Test System

Species:	Rabbit (*Oryctolagus cuniculus*)
Breed:	New Zealand White
Source:	Myrtle's Rabbitry Inc.
Sex:	Male
Body Weight Range:	3.2 kg to 3.5 kg
Age:	No particular age was prescribed for this test
Acclimation Period:	Minimum 5 days
Number of Animals:	Three
Identification Method:	Ear tag

Justification of Test System

The pyrogen test is specified in the current USP and ISO 10993, Part 11 guidelines. No *in vitro* alternative exists for detecting material mediated pyrogens.

4. Animal Management

Husbandry:	Conditions conformed to NAMSA Standard Operating Procedures that are based on the "*Guide for the Care and Use of Laboratory Animals.*"
Food:	Food was withheld from the animals used during the period of the test.
Water:	Potable water was provided *ad libitum* 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 animal number, test code, and sex. Noise level will be maintained to avoid exciting the animals.

Environment:	The room temperature and relative humidity were monitored daily. The temperature for the room was 20°C-23°C (68.0°F-73.4°F) and the relative humidity was 30-70%. The room was also free from disturbances likely to excite 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:	Rabbits used in this study were used previously for another pyrogen study. The establishment and maintenance of a pyrogen colony is defined in the current USP. Complete history of animal usage is traceable in laboratory records. The reuse criteria are described in NAMSA Standard Operating Procedures.
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

Animals were placed in a restrainer and allowed to acclimate for a minimum of 2 hours.

Preliminary Test - Sham
Animals were placed in a restrainer and a rectal probe was inserted in the rectum of each animal. The animals were allowed to acclimate to their restrainer position at least 1 hour prior to initiating temperature collection. Four temperature readings were conducted at 30 minute intervals beginning 90 minutes prior to injection. The mean temperature of the last two readings was considered the initial temperature.

Each of the rabbits was injected intravenously via the marginal ear vein with SNPS at a dose of 10 mL/kg. The solution was injected within a 4 minute period. For all rabbits, temperatures were recorded at 30 minute intervals for 3 hours after injection. Animals with a temperature rise of greater than 0.6°C, animals with temperatures outside of the 38.0°C to 39.8°C range or animals with a temperature variation greater than 0.2°C between the 0.5 and 0.0 hour pre-injection temperatures were excluded from the main test.

Main Test
Three animals were placed in a restrainer and a rectal probe was inserted in the rectum of each animal. The animals were allowed to acclimate to their restrainer position at least 15 minutes prior to initiating temperature collection. Two control temperatures were taken at least 30 minutes apart. The second temperature was recorded no more than 30 minutes prior to the injection; this temperature became the baseline temperature for the study.

Each of the rabbits was injected intravenously via the marginal ear vein with the test extract at 10 mL/kg of body weight. The test extract was injected within a 10 minute period. For all rabbits, temperatures were recorded at 30 minute intervals between 1 and 3 hours after injection.

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

No statistical analysis of the data was performed. Once the temperature readings had been recorded, the maximum rise in temperature for each rabbit was determined. A decrease in temperature was recorded as a 0.0°C change.–If no single animal showed an increase of 0.5°C or more above its baseline temperature, then the extract was judged nonpyrogenic. If the total maximum temperature of all three rabbits exceeded 3.3°C the extract was judged pyrogenic.

7. Results

Main Test

No single animal showed a temperature increase of 0.5°C or more above its baseline temperature. Individual temperatures are presented below.

Rabbit Number	Sex	Weight (kg)	Dose Volume (mL)	Temperature - °C							Maximum Rise
				Before Injection		Hours after Injection					
				Control 1	Baseline	1.0	1.5	2.0	2.5	3.0	
94592*	Male	3.5	35	39.2	39.2	39.1	39.1	39.2	39.1	39.1	0.0
94593*	Male	3.2	32	39.4	39.3	39.3	39.3	39.4	39.3	39.3	0.1
94594*	Male	3.3	33	39.6	39.7	39.6	39.5	39.6	39.5	39.8	0.1
										TOTAL RISE:	0.2

*Previous use history traceable in laboratory records.

8. Conclusion

Under the conditions of this study, the total rise of rabbit temperatures during the 3 hour observation period was within acceptable USP limits. The test article was judged as nonpyrogenic.

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. 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 was issued with the report.

10. Records

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

11. References

21 CFR 58 (GLP Regulations).

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.

International Organization for Standardization (ISO) 10993-11, Biological Evaluation of Medical Devices - Part 11: Tests for Systemic Toxicity (2006).

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

United States Pharmacopeia 32, National Formulary 27 (USP), General Chapter 151, Pyrogen Test (2009).

Phase Inspected	Date Inspected	Date Reported to Study Director	Date Reported to Management
Injections	October 26, 2009	October 26, 2009	October 26, 2009
Study Data Review	October 27, 2009	October 27, 2009	October 27, 2009
Final Report Review	October 30, 2009	October 30, 2009	October 30, 2009

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: _____ _____

William Wooten, ASQ-CQA
Quality Assurance

Date

GLP SAMPLE SUBMISSION FORM

C101609_040

UPS NAMSA OH

09C_48499

09T_46749

This form must be completed electronically using Micros 29229_001 29229 is form 29229_001 29229
Proposal. Upon receipt of these two documents, NAMS have any
Technical Specialist domestically at 1-866-666-9455 or internationally at 1-419-666-9455. Thank you for your business.

Ship To Information (final report will be mailed to this address)

Company Name:	IMBiotechnologies Ltd
Contact:	Mike Stewart
Address:	Suite 113 – Advanced 9650 20th Avenue
City, State, Zip:	Edmonton, Alberta T6N 1G1
Country:	Canada
Phone:	780-945-6609
Fax:	780-987-0941
E-mail:	mstewart@tbwifi.ca

Bill To Information

X Same as Ship To Information

Company Name:	
Address:	
City, State, Zip:	
Country:	
Phone (Accounts Payable):	

Reports should also be sent by (choose one): ☐ Fax X E-mail

Method of Payment (please choose one)

☐ Purchase Order: Purchase Order Number:

Cost Estimate Proposal Number: T09_4519

X Credit Card: Credit Card #: 5491395017125385 Credit Card Type: MC Please choose one if applicable Exp. Date: Nov 2010 Credit Card Holder Name: Michael Stewart

☐ Prepayment: Prepayment Amount: Prepayment Type: Please choose one if applicable

Test Article Description

Test Article Name:	Occlusin 505 Artificial Embolization Device
Test Article Identification:	Please choose one Enter Batch Code or Lot Number: FL288
Quantity Submitted:	4 vials
Test Article Sterilization:	X Sterilized ☐ Not Sterilized
Test Article Physical Description:	Microspheres supplied as a dry powder in sterile sealed glass vials; 400 mg/vial
Test Article Intended Clinical Use:	Embolotherapy
Test Article Type:	Please choose one If Other, please describe:

*A detailed composition list and current MSDS must accompany any chemical, pharmaceutical, or biologic. A certificate of testing or reprocessing must be submitted for any human-tissue-derived sample or clinically used medical device.

Optional Services (Additional fees will apply. Inquire for pricing.)

☐ Steam Sterilization: Test article autoclaved at 121°C

☐ EO Sterilization: Test article exposed to ethylene oxide (600 mg/L)

Storage Conditions

4 °C

Sample in CSI Refrigerator #_____

Test Article Disposition

After testing, NAMSA should:

X Discard unused test article

☐ Return unused test article

☐ Return used & unused test article

Method of Return Shipment

Please choose one if applicable Other:

Account #:

Test 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. Test Article Characterization definitions are available for download in pdf format on our website. Click this link http://www.namsa.com/clients/ to locate this pdf.

Test Article Stability

X Stability testing is in progress and sponsor affirms that test article is stable for duration of intended testing.

☐ Stability testing is complete and on file with sponsor. Expiration Date:

☐ Marketed product stability is characterized by its labeling. Expiration Date:

Test Article Mixture Analysis

X Analysis is not necessary because test article is a solid, powder, gel, or liquid being extracted or being tested as received (will not be mixed with a carrier).

☐ Sponsor will provide analytical methods.

☐ Sponsor will perform analysis on representative aliquots provided by NAMSA. Results must be provided to NAMSA.

Test Article Strength

X Strength is: 400 mg/vial

☑ Strength is not applicable because no active ingredients are used to formulate a concentration.

Test Article Purity

☐ Purity is:

X Purity is not applicable because the test article is a multi-component device.

Test Article Composition

Test article is composed of the following materials: polylactide –co-glycolide and bovine collagen

Testing: Biocompatibility/Chemistry

Test Code	Description	Time &Temp.*	Extractant(s)*
V0027_300	USP LAL		
V0022_000	C3a Complement		
V0639_000	C5b-9 Complement		
TU010_807	Pyrogen – Material Mediated		

Time/Temp. Key

A. 121°C/1 hour
B. 70°C/24 hours
C. 50°C/72 hours
D. 37°C/24 hours (cytotoxicity only)
E. 37°C/72 hours
F. 50°C/24 hours (hexane only)
G: Other (specify)

Extractant Key

A. Alcohol in Saline
B. Balanced Salt Solution
C. 1X MEM
D. Sesame/Vegetable Oil
E. Polyethylene Glycol
F. Purified Water
G. Sodium Chloride USP

REV072109

NAMSA

GLP SAMPLE SUBMISSION FORM

PEOPLE › SCIENCE › SOLUTIONS

09C-48499

www.namsa.com

Testing: Biocompatibility/Chemistry (con't...)

09T-46749

NAMSA recommends the highest temperature that will not degrade the test article unless other limits apply.

*For tests that require extraction only,

H. Isopropyl Alcohol
I. Hexane
J. CMF-PBS
K. DMSO

Special Instructions:

Product is biodegradable. Extraction temperature should be no more than 37 °C, where required.

Product is microspheres packaged as a dry sterile powder in sealed sterile glass vials. Each vial contains 400 mg of microspheres with an approximate product surface area of 2575 cm^2; or 6436 cm^2 per gram of product.

TEST FACILITY:

NAMSA
9 Morgan
Irvine, OH 92618

SPONSOR:

Mike Stewart
Suite 113 – Advanced 9650 20th Avenue
Edmonton, Alberta T6N 1G1
Canada

STUDY TITLE:

USP Pyrogen Study, Material Mediated

TABLE OF CONTENTS

09C-48499 02

Approvals

Sponsor Representative (Sponsor): M.W. Stewart

Date Approved: September 28, 2009

Study Director (NAMSA):

Date Initiated: 10-16-09

09C-48499 02

09T-46749 05

1. Introduction

Purpose
The purpose of the study is to determine if a test solution (TS) induces a pyrogenic response following intravenous injection in rabbits. The test article will be extracted in sterile, nonpyrogenic 0.9% sodium chloride solution (SNPS). This study will be conducted based on the methods described in USP General Chapter <151> PYROGEN TEST. The USP method is recommended by ISO 10993-11 (2006) Biological Evaluation of Medical Devices - Part 11: Tests for Systemic Toxicity.

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 was provided to NAMSA by the sponsor and is listed below:

Test Article Name:	Occlusin 505 Artificial Embolization Device
Test Article Identification:	FL288
Test Article Physical Description:	Microspheres supplied as a dry powder in sterile sealed glass vials/ 400mg/vial
Test Article Intended Clinical Use:	Embolotherapy
Test Article Stability:	Stability testing is in progress and sponsor affirms that test article is stable for duration of intended testing.
Test Article Strength:	Strength is not applicable because no active ingredients are used to formulate a concentration.
Test Article Purity:	Purity is not applicable because the test article is a multi-component device.
Test Article Composition:	The test article is composed of the following materials: polylactide – co-glycolide and bovine collagen
Test Article Mixture Analysis:	Analysis is not necessary because test article is a solid, powder, gel, or liquid being extracted or being tested as received (will not be mixed with a carrier).
Test Article Disposition:	Discard unused test article.

Preparation
The following information was completed based on the sponsor providing the information to NAMSA. Further instructions may be attached to the protocol. It is recommended that a minimum 150 mL extract of the material in SNPS be prepared as follows:

Ratio of Test Article to Extraction Vehicle
Material thickness less than 0.5 mm - 900 cm^2:150 mL (ratio of 120 cm^2:20 mL)

Test Article Preparation Instructions
Prepare based on the following: Use 0.14g of sample. 0.14g of sample has a surface area of 901.25cm^2.

Extraction Conditions
37°C, 72 hours

Does the test article contain a protein component?

Yes

The test solution will be warmed in a 37°C water bath or incubator for a minimum of 10 minutes prior to dosing.

Special Laboratory Instructions
Following the extraction procedure, it is acceptable to centrifuge the extract with the sample in it to be able to remove enough extract for testing.

Control Article
No control article will be needed in the study.

3. Test System

Test System

Species:	Rabbit (*Oryctolagus cuniculus*)
Breed:	New Zealand White
Source:	USDA licensed supplier
Sex:	No particular sex is prescribed for this test
Body Weight Range:	Not less than 1.5 kg at injection
Age:	No particular age is prescribed for this test
Acclimation Period:	Minimum 5 days
Number of Animals:	Three
Identification Method:	Ear tag

Justification of Test System
The pyrogen test is specified in USP General Chapter <151> Pyrogen Test and ISO 10993-11 (2006) Biological Evaluation of Medical Devices - Part 11: Tests for Systemic Toxicity. No *in vitro* alternative exists for detecting material mediated pyrogens. Three healthy albino rabbits, previously sham tested will be used for the study. Five additional rabbits may be required for retest in the event of a high temperature rise in the three rabbit test.

4. Animal Management

Husbandry:	Conditions will conform to NAMSA Standard Operating Procedures that are based on the "*Guide for the Care and Use of Laboratory Animals.*"
Food:	Food will be withheld from the animals used during the period of the test.
Water:	Potable water will be provided *ad libitum* through species appropriate water containers or delivered through an automatic watering system.
Contaminants:	Contaminants reasonably expected in feed or water supplies should not have the potential to influence the outcome of this test.
Housing:	Animals will be individually housed in stainless steel suspended cages identified by a card indicating the animal number, test code and sex. Noise level will be maintained to avoid exciting the animals.
Environment:	The room temperature will be monitored daily. The temperature range for the room is 20°C and 23°C (68.0°F and 73.4°F) and free from disturbances likely to excite the animals. The room humidity will be monitored daily. The humidity range for the room is 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 will be appropriately qualified and trained.
Selection:	Rabbits used in this study may have been used previously for another pyrogen study. The establishment and maintenance of a pyrogen colony is defined in the current USP. Complete history of animal usage will be traceable in laboratory records. The reuse criteria are described in NAMSA Standard Operating Procedures.
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:	All anesthetics, analgesics, and other medications may be given or altered at the discretion of the attending veterinarian in accordance with standard veterinary practice and the study objectives. This applies to specific medication, dose, and dosing intervals. 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 Guidelines 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. Method

Three animals will be placed in a restrainer and a rectal probe will be inserted in the rectum of each animal. The animals will be allowed to acclimate to their restrainer position at least 15 minutes prior to initiating temperature collection. Two control temperatures will be taken at least 30 minutes apart. The second temperature will be recorded no more than 30 minutes prior to the injection; this will become the baseline temperature for the study.

Each of the rabbits will be injected intravenously via the marginal ear vein with the test extract at 10 mL/kg of body weight. The TS will be injected within a 10 minute period. For all rabbits, temperatures will be recorded at 30 minute intervals between 1 and 3 hours after injection. After the test is completed, all animals will be handled in accordance with IACUC approved NAMSA procedures.

6. Evaluation and Statistical Analysis

No statistical analysis of the data will be performed. Once the temperature readings have been recorded, the maximum rise in temperature for each rabbit will be determined. If no single animal shows an increase of 0.5°C or more above its baseline temperature, then the extract will be judged nonpyrogenic. If the total maximum temperature of all three rabbits exceeds 3.3°C, the extract will be judged pyrogenic. If one or more rabbits show an increase of 0.5°C or more above its baseline temperature then the extract will be injected in five additional rabbits. In this case, if the total temperature increase of the eight rabbits does not exceed 3.3°C and if no more than three of the eight rabbits attain an individual temperature increase of 0.5°C or more, the extract will be judged nonpyrogenic. A decrease in temperature will be recorded as a 0.0°C change. In the case of a retest a fresh extract will be prepared or the refrigerated extract can be used, provided it does not exceed 24 hours after decanting.

If the test solution is judged pyrogenic, an *in vitro* endotoxin specific assay may be conducted to confirm that the response is due to a non-endotoxin (chemical). Approval by the sponsor will be obtained prior to the conduct of the *in vitro* assay.

7. Report

The final report will include a description of the methods employed, baseline and temperature recordings at 30 minute intervals and maximum temperature rises for the test animals.

8. 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.

9. 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).

10. Records

Test article preparation, body weights, rabbit temperatures and maximum rises 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.

09T-46749 05

11. References

Code of Federal Regulations (CFR), Title 21, Part 58, Good Laboratory Practice for Nonclinical Studies (2008).

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.

International Organization for Standardization (ISO) 10993-11 Biological Evaluation of Medical Devices - Part 11: Tests for Systemic Toxicity (2006).

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

United States Pharmacopeia 32, National Formulary 27 (USP), General Chapter 151, Pyrogen Test (2009).

12. 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

PEOPLE SCIENCE SOLUTIONS

USA Corporate Headquarters
6750 Wales Rd
Northwood, Ohio 43619
T 866.666.9455 (toll free)
F 419.662 4386

California
9 Morgan
Irvine, California 92618
T 949.951.3110
F 949.951.3280

Georgia
900 Circle 75 Parkway
Suite 1680
Atlanta, Georgia 30339
T 770 563.1660
F 770.563.1661

Ohio
6750 Wales Rd
Northwood, Ohio 43619
T 419.666.9455
F 419 666.2954

October 19, 2009

Michael Stewart
IMBiotechnologies LTD
Edmonton Research Park 9650 20th AvenueSuite 113 Advanved Technology Centre
Edmonton, AB, T6N 1G1
Canada

PROTOCOL AMENDMENT I

Test Article: Occlusin 505 Artificial Embolization Device

Identification: FL288

NAMSA Submission ID.: 09C_48499

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:
TU010_807	09C_48499_02	Pyrogen Study - Material Mediated- 0.9% SC Extract	October 19, 2009	November 09, 2009

Marcia Mestre, B.S.
Study Director

10-19-09
Date

cc: QA (NAMSA)
Sponsor

NAMSA

PEOPLE › SCIENCE › SOLUTIONS

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

California
9 Morgan
Irvine, California 92618
T 949.951.3110
F 949.951.3280

Georgia
900 Circle 75 Parkway
Suite 1680
Atlanta, Georgia 30339
T 770.563.1660
F 770.563.1661

Ohio
6750 Wales Rd
Northwood, Ohio 43619
T 419.666.9455
F 419.666.2954

October 30, 2009

Michael Stewart
IMBiotechnologies LTD
Suite 113 – Advanced 9650 20th Avenue
Edmonton, Alberta T6N 1G1
Canada

REVISED**
PROTOCOL AMENDMENT I

Test Article: Occlusin 505 Artificial Embolization Device

Identification: FL288

NAMSA Submission ID.: 09C_48499

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:
TU010_807	09C_48499_02	Pyrogen Study - Material Mediated- 0.9% SC Extract	October 19, 2009	November 09, 2009

**This amendment has been issued to correct the Sponsor facility address.

Marcia Mestre, B.S.
Study Director

10-30-09
Date

cc: QA (NAMSA)
Sponsor

GLP PROTOCOL

TEST FACILITY:

NAMSA
9 Morgan
Irvine, OH 92618

SPONSOR:

NAMSA Ohio
6750 Wales Road
Northwood, OH 43619

STUDY TITLE:

USP Pyrogen Study, Material Mediated

TABLE OF CONTENTS Page

Approvals

Sponsor Representative (Sponsor): _____

Date Approved: _____

Study Director (NAMSA): _____

Date Initiated: _____

1. Introduction

Purpose
The purpose of the study is to determine if a test solution (TS) induces a pyrogenic response following intravenous injection in rabbits. The test article will be extracted in sterile, nonpyrogenic 0.9% sodium chloride solution (SNPS). This study will be conducted based on the methods described in USP General Chapter <151> PYROGEN TEST. The USP method is recommended by ISO 10993-11 (2006) Biological Evaluation of Medical Devices - Part 11: Tests for Systemic Toxicity.

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 was provided to NAMSA by the sponsor and is listed below:

Test Article Name:	Occlusion 505 Artificial Embolization Device
Test Article Identification:	FL288
Test Article Physical Description:	Microspheres supplied as a dry powder in sterile sealed glass vials/ 400mg/vial
Test Article Intended Clinical Use:	Embolotherapy
Test Article Stability:	Stability testing is in progress and sponsor affirms that test article is stable for duration of intended testing.
Test Article Strength:	Strength is not applicable because no active ingredients are used to formulate a concentration.
Test Article Purity:	Purity is not applicable because the test article is a multi-component device.
Test Article Composition:	The test article is composed of the following materials: polylactide – co-glycolide and bovine collagen
Test Article Mixture Analysis:	Analysis is not necessary because test article is a solid, powder, gel, or liquid being extracted or being tested as received (will not be mixed with a carrier).
Test Article Disposition:	Discard unused test article.

Preparation
The following information was completed based on the sponsor providing the information to NAMSA. Further instructions may be attached to the protocol. It is recommended that a minimum 150 mL extract of the material in SNPS be prepared as follows:

Ratio of Test Article to Extraction Vehicle
Material thickness less than 0.5 mm - 900 cm^2:150 mL (ratio of 120 cm^2:20 mL)

Test Article Preparation Instructions
Prepare based on the following: Use 0.14g of sample. 0.14g of sample has a surface area of 901.25cm^2.

Extraction Conditions
37°C, 72 hours

Does the test article contain a protein component?

No

The test solution will be warmed in a 37°C water bath or incubator for a minimum of 10 minutes prior to dosing.

Special Laboratory Instructions
Following the extraction procedure, it is acceptable to centrifuge the extract with the sample in it to be able to remove enough extract for testing.

Control Article
No control article will be needed in the study.

3. Test System

Test System

Species:	Rabbit (*Oryctolagus cuniculus*)
Breed:	New Zealand White
Source:	USDA licensed supplier
Sex:	No particular sex is prescribed for this test
Body Weight Range:	Not less than 1.5 kg at injection
Age:	No particular age is prescribed for this test
Acclimation Period:	Minimum 5 days
Number of Animals:	Three
Identification Method:	Ear tag

Justification of Test System
The pyrogen test is specified in USP General Chapter <151> Pyrogen Test and ISO 10993-11 (2006) Biological Evaluation of Medical Devices - Part 11: Tests for Systemic Toxicity. No *in vitro* alternative exists for detecting material mediated pyrogens. Three healthy albino rabbits, previously sham tested will be used for the study. Five additional rabbits may be required for retest in the event of a high temperature rise in the three rabbit test.

4. Animal Management

Husbandry:	Conditions will conform to NAMSA Standard Operating Procedures that are based on the "*Guide for the Care and Use of Laboratory Animals*."
Food:	Food will be withheld from the animals used during the period of the test.
Water:	Potable water will be provided *ad libitum* through species appropriate water containers or delivered through an automatic watering system.
Contaminants:	Contaminants reasonably expected in feed or water supplies should not have the potential to influence the outcome of this test.
Housing:	Animals will be individually housed in stainless steel suspended cages identified by a card indicating the animal number, test code and sex. Noise level will be maintained to avoid exciting the animals.
Environment:	The room temperature will be monitored daily. The temperature range for the room is 20°C and 23°C (68.0°F and 73.4°F) and free from disturbances likely to excite the animals. The room humidity will be monitored daily. The humidity range for the room is 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 will be appropriately qualified and trained.
Selection:	Rabbits used in this study may have been used previously for another pyrogen study. The establishment and maintenance of a pyrogen colony is defined in the current USP. Complete history of animal usage will be traceable in laboratory records. The reuse criteria are described in NAMSA Standard Operating Procedures.
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: All anesthetics, analgesics, and other medications may be given or altered at the discretion of the attending veterinarian in accordance with standard veterinary practice and the study objectives. This applies to specific medication, dose, and dosing intervals. 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 Guidelines 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. Method

Three animals will be placed in a restrainer and a rectal probe will be inserted in the rectum of each animal. The animals will be allowed to acclimate to their restrainer position at least 15 minutes prior to initiating temperature collection. Two control temperatures will be taken at least 30 minutes apart. The second temperature will be recorded no more than 30 minutes prior to the injection; this will become the baseline temperature for the study.

Each of the rabbits will be injected intravenously via the marginal ear vein with the test extract at 10 mL/kg of body weight. The TS will be injected within a 10 minute period. For all rabbits, temperatures will be recorded at 30 minute intervals between 1 and 3 hours after injection. After the test is completed, all animals will be handled in accordance with IACUC approved NAMSA procedures.

6. Evaluation and Statistical Analysis

No statistical analysis of the data will be performed. Once the temperature readings have been recorded, the maximum rise in temperature for each rabbit will be determined. If no single animal shows an increase of 0.5°C or more above its baseline temperature, then the extract will be judged nonpyrogenic. If the total maximum temperature of all three rabbits exceeds 3.3°C, the extract will be judged pyrogenic. If one or more rabbits show an increase of 0.5°C or more above its baseline temperature then the extract will be injected in five additional rabbits. In this case, if the total temperature increase of the eight rabbits does not exceed 3.3°C and if no more than three of the eight rabbits attain an individual temperature increase of 0.5°C or more, the extract will be judged nonpyrogenic. A decrease in temperature will be recorded as a 0.0°C change. In the case of a retest a fresh extract will be prepared or the refrigerated extract can be used, provided it does not exceed 24 hours after decanting.

If the test solution is judged pyrogenic, an *in vitro* endotoxin specific assay may be conducted to confirm that the response is due to a non-endotoxin (chemical). Approval by the sponsor will be obtained prior to the conduct of the *in vitro* assay.

7. Report

The final report will include a description of the methods employed, baseline and temperature recordings at 30 minute intervals and maximum temperature rises for the test animals.

8. 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.

9. 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).

10. Records

Test article preparation, body weights, rabbit temperatures and maximum rises 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.

11. References

Code of Federal Regulations (CFR), Title 21, Part 58, Good Laboratory Practice for Nonclinical Studies (2008).

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.

International Organization for Standardization (ISO) 10993-11 Biological Evaluation of Medical Devices - Part 11: Tests for Systemic Toxicity (2006).

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

United States Pharmacopeia 32, National Formulary 27 (USP), General Chapter 151, Pyrogen Test (2009).

12. 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.