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:

Cytotoxicity Study Using the ISO Elution Method (1X MEM Extract)

TEST ARTICLE:

Occlusin 500 Artificial Embolization Device

IDENTIFICATION NO.:

Batch: FL288

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Summary

An *in vitro* biocompatibility study, based on the International Organization for Standardization 10993: Biological Evaluation of Medical Devices, Part 5: Tests for Cytotoxicity: *in vitro* Methods guidelines, was conducted on the test article, Occlusin 500 Artificial Embolization Device, Batch: FL288, to determine the potential for cytotoxicity. A single extract of the test article was prepared using single strength Minimum Essential Medium supplemented with 5% serum and 2% antibiotics (1X MEM). This test extract was placed onto three separate monolayers of L-929 mouse fibroblast cells propagated in 5% CO_2. Three separate monolayers were prepared for the reagent control, negative control and for the positive control. All monolayers were incubated at 37°C in the presence of 5% CO_2 for 48 hours. The monolayer in the test, reagent control, negative control and positive control wells was examined microscopically at 48 hours to determine any change in cell morphology.

Under the conditions of this study, the 1X MEM test extract showed no evidence of causing cell lysis or toxicity. The 1X MEM test extract met the requirements of the test since the grade was less than a grade 2 (mild reactivity). The reagent control, negative control and the positive control performed as anticipated.

Study and Supervisory Personnel:

Molly F. Corvo, B.S.
Scott A. Summers
Heatherbea L. Weirich, B.S.
Jennifer N. Moritz, B.S.
Debra S. Dunn

Approved by:

Michelle E. Longstreet

Michelle E. Longstreet, B.S.
Study Director

6-22-07
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

Michelle E. Longstreet, B.S.

6-22-07

Date

1. Introduction

Purpose
The test article identified below was extracted, and the extract was subjected to an *in vitro* cytotoxicity study for biocompatibility based on the requirements of the International Organization for Standardization 10993: Biological Evaluation of Medical Devices, Part 5: Tests for Cytotoxicity: *in vitro* Methods. The test was performed to determine whether leachables extracted from the material would cause cytotoxicity.

Dates
The test article was received on May 17, 2007. The cells were first exposed to the extract June 8, 2007, and the observations were concluded on June 10, 2007.

GLP Compliance
The study initiated by protocol signature on May 30, 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.

2. Materials

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

Test Article:	Occlusin 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 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.
Physical Description of Test Article:	Dry white polymer beads ~400 µm
Storage Conditions:	Refrigerated
Extraction Vehicle:	Single strength Minimum Essential Medium supplemented with 5% serum and 2% antibiotics (1X MEM)
Test Article Preparation:	The test article was prepared based on the sponsor supplied surface area of 44 cm^2 per sample. Two samples were included in the preparation. Based on the USP ratio of 120 cm^2:20 ml, a 88.0 cm^2 portion of the test article was covered with 15 ml of 1X MEM. Each vial was filled with 7.5 ml of extract to remove the test article from the container. A single preparation was extracted with agitation at 37°C for 24 hours.
Negative Control Preparation:	High density polyethylene was used as the negative control. Based on the USP ratio of 60 cm^2:20 ml, a single 30.8 cm^2 portion of the control material was covered with 10 ml of 1X MEM. The preparation was subjected to the extraction conditions previously described for the test article.
Reagent Control Preparation:	A single aliquot of 1X MEM without test material was subjected to the same extraction conditions as described for the test article.
Positive Control Preparation:	The current NAMSA positive control, tin stabilized polyvinylchloride, was used. Based on the USP ratio of 60 cm^2:20 ml, a single 60.8 cm^2 portion of the control material was covered with 20 ml of 1X MEM and extracted with agitation at 37°C for 24 hours.

Condition of Extracts:

Test: clear
Reagent Control: clear
Negative Control: clear
Positive Control: clear

3. Test System

Test System Management

L-929, mouse fibroblast cells, (ATCC CCL 1, NCTC Clone 929, of strain L, or equivalent source) were propagated and maintained in open wells containing single strength Minimum Essential Medium supplemented with 5% serum and 2% antibiotics (1X MEM) in a gaseous environment of 5% carbon dioxide (CO_2). For this study, 10 cm^2 wells were seeded, labeled with passage number and date, and incubated at 37°C in 5% CO_2 to obtain sub-confluent monolayers of cells prior to use. Aseptic procedures were used in the handling of the cell cultures following approved NAMSA Standard Operating Procedures.

4. Methods

Triplicate culture wells were selected which contained a sub-confluent cell monolayer. The growth medium contained in triplicate cultures was replaced with 2 ml of the test extract. Similarly, triplicate cultures were replaced with 2 ml of the reagent control, negative control and the positive control. Each well was labeled with the corresponding lab number, replicate number and the dosing date. The wells were incubated at 37°C in 5% CO_2 for 48 hours.

Following incubation, the cultures were examined microscopically (100X) to evaluate cellular characteristics and percent lysis. The color of the test medium was observed. A color shift toward yellow was associated with an acidic pH range and a color shift toward magenta to purple was associated with an alkaline pH range.

Each culture well was evaluated for percent lysis and cellular characteristics using the following table (direct excerpt from USP):

Grade	Reactivity	Conditions of all Cultures
0	None	Discrete intracytoplasmic granules; no cell lysis
1	Slight	Not more than 20% of the cells are round, loosely attached, and without intracytoplasmic granules; occasional lysed cells are present
2	Mild	Not more than 50% of the cells are round and devoid of intracytoplasmic granules; no extensive cell lysis and empty areas between cells
3	Moderate	Not more than 70% of the cell layers contain rounded cells or are lysed
4	Severe	Nearly complete destruction of the cell layers

For the test to be valid, the reagent control and the negative control must have had a reactivity of none (grade 0) and the positive control must have been a grade 3 or 4. The test sample met the requirements of the test if the biological response was less than or equal to grade 2 (mild). The test would have been repeated if the controls did not perform as anticipated and/or if all three test wells did not yield the same conclusion.

5. Results

See Appendix 1 for results.

pH Observation: No pH shift observed at 48 hours.

6. Conclusion

Under the conditions of this study, the 1X MEM test extract showed no evidence of causing cell lysis or toxicity. The 1X MEM test extract met the requirements of the test since the grade was less than a grade 2 (mild reactivity). The reagent control, negative control and the positive control performed as anticipated.

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 ISO 13485:2003.

7. 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.

8. 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).

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

21 CFR 58 (GLP Regulations).

ISO 10993-5 (1999) Biological evaluation of medical devices – Part 5: Tests for cytotoxicity, *in vitro* methods.

USP 29 – NF 24 General Chapter <87> BIOLOGICAL REACTIVITY TESTS, IN VITRO.

Wilsnack, R. E., "Quantitative Cell Culture Biocompatibility Testing of Medical Devices and Correlation to Animal Tests," *Biomaterials, Medical Devices and Artificial Organs* 4 (1976): 235-261.

Wilsnack, R. E., F. J. Meyer and J. G. Smith, "Human Cell Culture Toxicity Testing of Medical Devices and Correlation to Animal Tests," *Biomaterials, Medical Devices and Artificial Organs* 1 (1973): 543-562.

11. 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 - Reactivity Grades For Elution Testing

Well	Percent Rounding	Percent Cells Without Intracytoplasmic Granules	Percent Lysis	Grade	Reactivity
Test (1A)	0	0	0	0	None
Test (1B)	0	0	0	0	None
Test (1C)	0	0	0	0	None
Negative Control (1A)	0	0	0	0	None
Negative Control (1B)	0	0	0	0	None
Negative Control (1C)	0	0	0	0	None
Reagent Control (1A)	0	0	0	0	None
Reagent Control (1B)	0	0	0	0	None
Reagent Control (1C)	0	0	0	0	None
Positive Control (1A)	100	100	100	4	Severe
Positive Control (1B)	100	100	100	4	Severe
Positive Control (1C)	100	100	100	4	Severe

Statement of Quality Assurance Activities

Phase Inspected	Auditor	Date
Dosing	K. J. Evener	June 8, 2007
Final Report Review	L. M. Byrd	June 22, 2007

Reports to Management and Study Director(s)	Date
Periodic Status Report	June 8, 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: Lisa M Byrd 6-22-07

Lisa M. Byrd, RQAP-GLP, CQA, ALAT
Auditor, Quality Assurance

Date

STORE IN REFRIGERATOR (+4°C)

CALIBRATION #: 7420

TECH/DATE: JS 5-17-07

LP SAMPLE SU

07T_36169

25447_001 25447

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SPONSOR FINAL REPORT WILL BE ADDRESSED AND MAILED TO

COMPANY NAME* VIREXX MEDICAL CORP. **ATTN*** PAUL TIEGE

ADDRESS* 8223 Roper Rd

CITY* Edmonton **STATE*** Alberta **ZIP*** T6E 6S4

COUNTRY* CANADA

PHONE* 780 989 6715

FAX* 780 436 0068

E-MAIL* ptiege@virexx.com

INVOICE INFORMATION

BILLING ADDRESS (include Company Name if different from mailed to)* VIREXX MEDICAL CORP.

PURCHASE ORDER NUMBER* V0725-185PT

COST ESTIMATE AND PROPOSAL NUMBER T07-2440 and T07_2708

☐ VISA ☐ MasterCard ☐ American Exp.

CARD HOLDER NAME

CREDIT CARD NUMBER 780 989 6721 **EXPIRATION DATE** 780 436 0068

ACCOUNTS PAYABLE PHONE* **ACCOUNTS PAYABLE FAX***

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

INTENDED CLINICAL USE OF TEST ARTICLE:*

☑ BATCH ☐ CODE ☐ LOT **CHECK ONE** FL288 **IDENTIFICATION NUMBER***

CONTROL ARTICLE NAME* N/A

☐ BATCH ☐ CODE ☐ LOT **CHECK ONE** **IDENTIFICATION NUMBER***

NAMSA recommends only one lot, batch, or code per test article submission.

QUANTITY SUBMITTED:* 8 vials Occlusion 500 (please specify quantities for each lot/batch/code provided)

PHYSICAL DESCRIPTION OF TEST ARTICLE (Chemical/Material type/Color)* dry white polymer beads ~ 400 µm

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 ☑ 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)
☑	☐	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.
☑	☐	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:**

(AUTHORIZED BY SPONSOR) Paul Michelle L. Longstreth **DATE** 15 MAY 07

NAMSA STUDY DIRECTOR **DATE** 5-29 incorrect date 5-30-07 MEL 5-30-07

T051707_021 FEDEX VIREXX Received 5-17-07

GLP PROTOCOL

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:

Cytotoxicity Study Using the ISO Elution Method

109935

Approvals

Sponsor Representative (Sponsor): PAUL TIEGE

Date Approved: 14 MAY 07

Study Director (NAMSA): Michelle E. Longstreth

Date Initiated: 5-30-07

Extraction Conditions (select one):

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

* The preferable extraction condition is 37°C for 24 hours using 1X MEM to simulate physiological conditions. At temperatures greater than 37°C, 1X MEM cannot be used.

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

✓ Discard ___ Return unused article ___ Return unused and used article

Special Laboratory Instructions:

Control Article
Negative Control: High density polyethylene, will be prepared based on a ratio of 60 cm^2:20 ml extraction vehicle. A single preparation of the material will be extracted using the same conditions as described for the test article.

Reagent Control: A single aliquot of the extraction vehicle without test material will be prepared using the same conditions as described for the test article.

Positive Control: Current NAMSA positive control material, tin stabilized polyvinylchloride, will be prepared based on a ratio of 60 cm^2:20 ml extraction vehicle. A single preparation of the material will be made and extracted at 37°C for 24 hours.

3. Test System

Test System and Justification
Mammalian cell culture monolayer, L-929, mouse fibroblast cells, (ATCC CCL 1, NCTC Clone 929, of strain L, or equivalent source), will be used. *In vitro* mammalian cell culture studies have been used historically to evaluate cytotoxicity of biomaterials and medical devices (Wilsnack, *et al.*, 1973).

Test System Management
L-929, mouse fibroblast cells, (ATCC CCL 1, NCTC Clone 929, of strain L, or equivalent source) will be propagated and maintained in open wells containing single strength Minimum Essential Medium supplemented with 5% serum and 2% antibiotics (1X MEM) in a gaseous environment of 5% carbon dioxide (CO_2). For this study, 10 cm^2 wells will be seeded, labeled with passage number and date, and incubated at 37°C in 5% CO_2 to obtain sub-confluent monolayers of cells prior to use. Aseptic procedures will be used in the handling of the cell cultures following approved NAMSA Standard Operating Procedures.

4. Method

Each culture well will be selected which contains a sub-confluent cell monolayer. The growth medium in triplicate cultures will be replaced with 2 ml of the test extract. Similarly, triplicate cultures will be replaced with 2 ml of the reagent, negative and positive control extracts. Each well will be labeled with the corresponding lab number, replicate number and the dosing date and incubated at 37°C in 5% CO_2 for 48 hours.

Following incubation, the cultures will be examined microscopically (100X) to evaluate cellular characteristics and percent lysis.

① completed by sponsor MEL 5-30-07

11. 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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May 31, 2007

Paul Tiege
ViRexx Medical Corporation
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Edmonton, Alberta, T6E 6S4 Canada

PROTOCOL AMENDMENT I

Test Article: Occlusin 500 Artificial Embolization Device

Identification: Batch: FL288

NAMSA Submission ID.: 07T_36169

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:
V0015_110	07T_36169_02	Cytotoxicity Study Using the ISO Agarose Overlay Method	June 4, 2007	June 21, 2007
V0014_130	07T_36169_03	Cytotoxicity Study Using the ISO Elution Method - 1X MEM Extract	June 4, 2007	June 22, 2007
T0625_500	07T_36169_04	ISO Systemic Toxicity Study - Extract - 0.9% SC Extract	June 4, 2007	June 28, 2007
T0625_500	07T_36169_05	ISO Systemic Toxicity Study - Extract - SO Extract	June 4, 2007	June 28, 2007

Michelle E. Longstreet

Michelle E. Longstreet, B.S.
Study Director

6-4-07
Date

cc: QA (NAMSA)
GLP study file

NAMSA

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June 21, 2007

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

PROTOCOL AMENDMENT II

Test Article:	Occlusin 500 Artificial Embolization Device
Identification:	Batch: FL288
Protocol:	V0014_130 Cytotoxicity Study Using the ISO Elution Method – 1X MEM Extract
NAMSA Lab No.:	07T_36169_03
Protocol:	T0625_500 ISO Systemic Toxicity Study – 0.9% SC, SO Extracts
NAMSA Lab No.:	07T_36169_04, 05

This amendment has been written to provide additional instructions to the **Preparation** section of the study protocols:

- Add the extract vehicle to the sponsor provided vials to remove the test article. Transfer the test article and extract to appropriate container for extraction.

This amendment to the protocol was written prior to testing. A copy of the original amendment is contained within the study file. This version serves as formal documentation of the amendment; it accurately reflects the content of the original amendment documentation.

Michelle E. Longstreet

Michelle E. Longstreet, B.S.
Study Director

6-21-07

Date

cc: QA (NAMSA)
GLP study file