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:

Genotoxicity: Bacterial Reverse Mutation Study (Soluble Material - DMSO)

TEST ARTICLE:

Occlusion 500 Artifical Embolization Device

IDENTIFICATION NO.:

Batch: FL288

TABLE OF CONTENTS Page

Summary

A *Salmonella typhimurium* and *Escherichia coli* reverse mutation standard plate incorporation study was conducted to evaluate whether a dimethyl sulfoxide test article solution of Occlusion 500 Artifical Embolization Device, Batch: FL288, would cause mutagenic changes in the average number of revertants for histidine-dependent *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537, and in tryptophan-dependent *Escherichia coli* strain WP2*uvrA* in the presence and absence of S9 metabolic activation. This study was conducted to satisfy, in part, the genotoxicity requirement of the International Organization for Standardization: Biological Evaluation of Medical Devices, Part 3: Tests for Genotoxicity, Carcinogenicity and Reproductive Toxicity.

The dimethyl sulfoxide test article solution was found to be noninhibitory to growth of tester strains TA98, TA100, TA1535, TA1537, and WP2*uvrA*. Separate tubes containing 2 ml of molten top agar supplemented with histidine-biotin solution for the *S. typhimurium* strains and with tryptophan for the *E. coli* strain were inoculated with 0.1 ml of culture for each of five tester strains, and 0.1 ml of the dimethyl sulfoxide test article solution. A 0.5 ml aliquot of sterile Water for Injection or S9 homogenate, simulating metabolic activation, was added when necessary. The mixture was poured across triplicate Minimal E plates. Parallel testing was also conducted with a negative control and five positive controls. The mean number of revertants of the triplicate test plates was compared to the mean number of revertants of the triplicate negative control plates for each of the five tester strains employed. The means obtained for the positive controls were used as points of reference.

Under the conditions of this assay, the dimethyl sulfoxide test article solution was considered to be nonmutagenic to *Salmonella typhimurium* tester strains TA98, TA100, TA1535, and TA1537, and to *Escherichia coli* strain WP2*uvrA*. The negative and positive controls performed as anticipated. The results of this study should be evaluated in conjunction with other required tests as listed in ISO 10993, Part 3: Tests for Genotoxicity, Carcinogenicity and Reproductive Toxicity.

Study and Supervisory Personnel:

Erica G. Kujawa, B.S.
Jennifer N. Moritz, B.A.
Shelly L. Nielsen, B.S.
Chandramallika Ghosh, Ph.D., DABT
Heather A. Huseman, B.S.

Approved by:

Michelle E. Longstreet

Michelle E. Longstreet, B.S.
Study Director

7-9-07
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.

7-9-07

Date

1. Introduction

Purpose
A *Salmonella typhimurium* and *Escherichia coli* reverse mutation standard plate incorporation study was conducted to evaluate whether a dimethyl sulfoxide test article solution would cause mutagenic changes in the average number of revertants for *Salmonella typhimurium* tester strains TA98, TA100, TA1535, and TA1537, and *Escherichia coli* tester strain WP2*uvrA* in the presence and absence of S9 metabolic activation. This test was conducted to satisfy, in part, the requirements of the International Organization for Standardization (ISO) 10993, Part 3: Tests for Genotoxicity, Carcinogenicity and Reproductive Toxicity. Bacterial reverse mutation tests have been widely used as rapid screening procedures for the determination of mutagenic and potential carcinogenic hazards.

Dates
The test article was received on May 24, 2007. The preliminary toxicity screen began on June 12, 2007, and the testing ended on June 15, 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 Certificate of Quality Assurance Inspections was issued with this report.

2. Materials

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

Test Article:	Occlusion 500 Artifical 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:	White beads
Storage Conditions:	Refrigerated
Vehicle:	Dimethyl sulfoxide (DMSO)
Preparation:	A 1.0 ml portion of the test article was mixed with DMSO up to 10 ml. A negative control (vehicle without test material) was similarly prepared.

	Test	Control
Condition of Solutions:	clear and pale yellow in color*	clear

*The test solution formed a precipitate when it was added to the agar.

3. Test System

Test System

Each *Salmonella typhimurium* tester strain contains a specific mutation in the histidine operon and other mutations that increase their ability to detect mutagens. In addition, the *Escherichia coli* contains a specific mutation in the tryptophan operon, and a deletion in the *uvrA* gene. These genetically altered *S. typhimurium* strains (TA98, TA100, TA1535, and TA1537) and *E. coli* strain (WP2*uvrA*) cannot grow in the absence of histidine or tryptophan, respectively. When placed in a histidine-free (for *S. typhimurium*) or tryptophan-free (for *E. coli*) medium, only those cells which mutate spontaneously back to their wild type state (histidine independent by manufacturing their own histidine, or tryptophan independent by manufacturing their own tryptophan) are able to form colonies. The spontaneous mutation rate (or reversion rate) for any one strain is relatively constant, but if a mutagen is added to the test system, the mutation rate is significantly increased.

Tester Strain	Mutations/Genotypic Relevance
S. typhimurium TA98	hisD3052, *rfa, uvrB*, frameshift, pKM101
S. typhimurium TA100	hisG46, *rfa*, *uvrB,* missense, pKM101
S. typhimurium TA1535	hisG46, *rfa*, *uvrB*, missense
S. typhimurium TA1537	hisC3076, *rfa*, *uvrB*, frameshift
E. coli WP2*uvrA*	trpE65, *uvrA*, missense

rfa	=	causes partial loss of the lipopolysaccharide wall which increases permeability of the cell to large molecules (i.e., crystal violet inhibition)
uvrB or *uvrA*	=	deficient DNA excision - repair system (i.e., ultraviolet sensitivity)
frameshift	=	base-pair addition/deletion
missense	=	base-pair substitution
pKM101	=	plasmid confers ampicillin resistance (R-factor) and enhances sensitivity to mutagens

Metabolic Activation

Aroclor 1254 - induced rat liver (S9 homogenate) was used as metabolic activation. The S9 homogenate is prepared from male, Sprague Dawley rats. The rats are induced with one intraperitoneal injection of Aroclor 1254 (500 mg/ml) 5 days prior to sacrifice. Just prior to use, the S9 homogenate was mixed with a buffer containing 0.4 M $MgCl_2$/1.65 M KCl, 1.0 M Glucose-6-phosphate, 0.1 M NADP, 0.2 M sodium phosphate buffer, and sterile Water for Injection.

Preparation of Tester Strains

Cultures of *Salmonella typhimurium*, TA98, TA100, TA1535 and TA1537, and *Escherichia coli*, WP2*uvrA*, were inoculated to individual Erlenmeyer flasks containing oxoid broth. The inoculated broth cultures were incubated at 37 ± 2°C in an incubator shaker operating at 115-125 rpm for 10-12 hours.

Negative Control

DMSO (vehicle without test material) was tested with each tester strain to determine the spontaneous reversion rate. Each strain was tested with and without S9 activation. These data represented a base rate to which the number of revertant colonies that developed in each test plate were compared to determine whether the test article had significant mutagenic properties.

Positive Control

A known mutagen, Dexon (paradimethylaminobenzene diazosulfonic acid sodium salt), was used as a positive control to demonstrate that tester strains TA98, TA100, and TA1537 were sensitive to mutation to the wild type state. For tester strain TA1535, sodium azide was used as a positive control. For tester strain TA100, 2-aminofluorene was also used as a positive control. For tester strain WP2*uvrA*, 2-aminoanthracene and methylmethane-sulfonate were used as positive controls. Although metabolic activation was only required with 2-aminofluorene and 2-aminoanthracene to induce mutagenic results, all positive controls were tested with and without S9 homogenate.

Strain Characteristics and Strain Standard Plate Counts

Strain characteristics were verified and viable counts were determined.

Spot Plate Inhibition Screen

The DMSO test article solution was evaluated by a spot plate technique, modeled after the antimicrobial zone of inhibition test. This screen was used to evaluate the toxicity of the solution to determine whether dilution of the solution was required to provide a solution noninhibitory to the *Salmonella typhimurium* or to the *Escherichia coli*.

Separate tubes containing 2 ml of molten top agar supplemented with histidine-biotin solution for the *Salmonella typhimurium* and tryptophan for the *Escherichia coli* were inoculated with 0.1 ml of culture for each of the five tester strains. After mixing, the agar was poured across the surface of separate Minimal E plates labeled with lab number and appropriate tester strain. Once the agar solidified, sterile filter discs were placed in the center of the plates. A 0.1 ml aliquot of the DMSO test article solution was added to the filter discs on each of the labeled plates. Parallel testing was conducted with a negative control, and to demonstrate a positive zone of inhibition, 10X Dexon was utilized.

The plates were incubated at 37°C for 2-3 days. Following the incubation period, the zone of growth inhibition was observed and recorded. Only solutions that were noninhibitory to the tester strains were tested by the standard plate incorporation method.

Sterility Verification Test
The sterility of the test article and positive and negative controls was verified using a sterility verification test. A 0.1 ml aliquot of the test article, negative control and each positive control was transferred to nutrient agar plates. In addition, a 0.1 ml aliquot of SWFI, S9 homogenate, histidine/biotin supplemented top agar, and tryptophan supplemented top agar were also added to nutrient agar plates. The plates were incubated at 37°C for 2 days and then evaluated for sterility.

4. Method

Standard Plate Incorporation Assay
Separate tubes containing 2 ml of molten top agar supplemented with histidine-biotin solution for the S*almonella typhimurium* or with tryptophan for the *Escherichia coli* were inoculated with 0.1 ml of culture for each of the five tester strains and 0.1 ml of the DMSO test article solution. A 0.5 ml aliquot of SWI or S9 homogenate, simulating metabolic activation, was added when necessary. The mixture was poured across triplicate Minimal E plates labeled with lab number, appropriate tester strain, and S9 metabolic activation (when applicable). Parallel testing was also conducted with a negative control and five positive controls.

Histidine-free media plates (for *S. typhimurium*) and tryptophan-free media plates (for *E. coli*) were prepared in triplicate as follows:

1. DMSO test article solution with and without S9 activation
2. Negative control with and without S9 activation
3. 1X Dexon (known mutagen) with and without S9 activation with strains TA98, TA100, and TA1537
4. 1X 2-Aminofluorene (known mutagen) with and without S9 activation with strain TA100
5. 1X Sodium azide (known mutagen) with and without S9 activation with strain TA1535
6. 1X 2-Aminoanthracene (known mutagen) with and without S9 activation with strain *WP2uvrA*
7. 1X Methylmethane-sulfonate (known mutagen) with and without S9 activation with strain WP2*uvrA*

The plates were incubated at 37°C for 2 days. Following the incubation period, the revertant colonies on each plate were recorded. The mean number of revertants was determined. The mean number of revertants of the test plates were compared to the mean number of revertants of the negative control for each of the five tester strains employed.

5. Evaluation

For the DMSO test article solution to be evaluated as a test failure or "potential mutagen" there must have been a 2-fold or greater increase in the number of mean revertants over the means obtained from the negative control for any or all five tester strains. Each positive control mean must have exhibited at least a 3-fold increase over the respective negative control mean of the *Salmonella* tester strain employed, and at least a 2-fold increase over the respective negative control mean of the *E. coli* tester strain. Exceptions included conditions not intended to provoke a mutagenic response (e.g. 2-aminoanthracene and 2-aminofluorene without metabolic activation). The negative control results of each tester strain exhibited a characteristic number of spontaneous revertants based on historical data collected at NAMSA.

6. Results

Strain Characteristics and Strain Standard Plate Count
Salmonella typhimurium strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* strain WP2*uvrA* exhibited appropriate genetic characteristics pertaining to this assay (see Appendix 1).

Spot Plate Inhibition Screen
No significant inhibition was observed (see Appendix 2).

Sterility Verification Test
The test article extract, negative control and each positive control were sterile.

Standard Plate Incorporation Assay
The results are summarized in Appendix 3. In no case was there a 2-fold or greater increase in the mean number of revertants of tester strains TA98, TA100, TA1535, TA1537, and WP2*uvrA* in the presence of a DMSO test article solution. The test article solution without S9 showed a mean revertant number below the range specified for strain WP2*uvrA*. However, the revertant number was within the historical range for spontaneous revertants for this strain. Thus, the mean is acceptable. Each positive control mean exhibited at least a 3-fold increase over the respective mean of the *S. typhimurium* tester strain employed and at least a 2-fold increase over the respective mean of the *E. coli* tester strain.

Test Validity
The data obtained from this study met NAMSA criteria for a valid assay.

7. Conclusion

Under the conditions of this assay, the dimethyl sulfoxide test article solution was considered to be nonmutagenic to *Salmonella typhimurium* tester strains TA98, TA100, TA1535, and TA1537, and to *Escherichia coli* strain WP2*uvrA*. The negative and positive controls performed as anticipated. This test was conducted to satisfy, in part, the requirements of the International Organization for Standardization (ISO) 10993, Part 3: Tests for Genotoxicity, Carcinogenicity and Reproductive Toxicity.

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.

8. 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 Certificate of Quality Assurance Inspections is provided with this final report.

9. 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).

10. Records

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

11. References

21 CFR 58 (GLP Regulations).

Ames, B.N., McCann, J., and Yamasaki, E., "Methods for Detecting Carcinogens and Mutagens with the Salmonella/Mammalian-Microsome Mutagenicity Test," Mutation Research 31, (1975): 347-364.

Brusick, D.J., V.F. Simmon, H.S. Rosenkranz, V.A. Ray, and R.S. Stafford, "An Evaluation of the *Escherichia coli* WP2 and WP2*uvrA* Reverse Mutation Assay," Mutation Research 76, (1980): 169-190.

Maron, Dorothy M., Ames, Bruce N., "Revised Methods for the Salmonella Mutagenicity Test," Mutation Research, 113 (1983): 175-215.

ISO 10993-3 (2003) Biological evaluation of medical devices - Part 3: Tests for genotoxicity, carcinogenicity and reproductive toxicity.

OECD Guideline for the Testing of Chemicals, Proposal for Replacement of Guidelines 471 Bacterial Reverse Mutation Test, Document Number 471.

Ortiz, A.I., M.T. Pollastrini, M. Barea, and D. Ordóñez, "Bacterial Mutagenic Evaluation of Luxabendazole, a New Broad Spectrum Antihelminic, with the *Salmonella typhimurium* Histidine and the *Escherichia coli* Tryptophan Reversions Tests," *Mutagenesis* 11 (1996): 27-31.

Test validation, Bacterial Mutagenicity Test: NAMSA lab number 98T-00785-00.

12. 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 - Strain Characteristics And Strain Standard Plate Counts

Characteristics (expected)	Tester Strains				
	TA98	TA100	TA1535	TA1537	WP2*uvrA*
Ampicillin-TA98 & TA100 = (Resistant) TA1535, TA1537 & WP2*uvrA* = (Sensitive)	R	R	S	S	S
rfa Mutation; CV (Sensitive)	S	S	S	S	NA
uvrB/*uvrA* (No Growth)	NG	NG	NG	NG	NG
Histidine Requirement; (Growth)	G	G	G	G	NA
Tryptophan Requirement; (Growth)	NA	NA	NA	NA	G
Biotin (No Growth)	NG	NG	NG	NG	NA
L-tryptophan (No Growth)	NA	NA	NA	NA	NG
Purity (Pure)	PURE	PURE	PURE	PURE	PURE
Total Plate Count CFU's (10^{-7})	96 94	144 165	347 321	86 76	294 335
Mean	95	155	334	81	315
Titer (Organisms/ml)	9.5×10^8	1.6×10^9	3.3×10^9	8.1×10^8	3.2×10^9

R = Resistant S = Sensitive NG = No Growth G = Growth NA = Not Applicable

Appendix 2 - Spot Plate Inhibition Screen Results

	Zone of Inhibition (mm)				
	TA98	TA100	TA1535	TA1537	WP2*uvrA*
DMSO negative control	0	20	0	19	15
DMSO test article solution	16	17	17	17	18
Dexon positive control	34	45	58	25	30

Appendix 3 - Standard Plate Incorporation Assay - Reversion Rates For Tester Strains

	Salmonella typhimurium								*Escherichia coli*	
	TA98		TA100		TA1535		TA1537		WP2*uvrA*	
	CFTP	Mean	CFTP	Mean	CFTP	Mean	CFTP	Mean	CFTP	Mean
DMSO w/o S9 negative control	23 23 15	20	132 119 122	124	27 33 19	26	3 5 7	5	25 11 24	20
DMSO w/ S9 negative control	29 11 19	20	150 165 150	155	22 25 32	26	12 8 12	11	19 25 19	21
DMSO test article solution w/o S9	17 14 19	17	144 113 124	127	19 10 17	15	6 3 3	4	19 18 19	19
DMSO test article solution w/ S9	14 28 16	19	133 134 129	132	20 23 32	25	3 4 4	4	27 25 22	25
Dexon w/o S9 positive control	1760 976 1024	1253	1088 1344 1376	1269			512 1072 496	693		
Dexon w/ S9 positive control	992 1232 992	1072	592 656 1232	827			496 880 880	752		
2-aminofluorene w/o S9 positive control*			258 215 227	233						
2-aminofluorene w/ S9 positive control†			2144 2122 1536	1931						
Sodium azide w/o S9 positive control					3920 3552 3472	3648				
Sodium azide w/ S9 positive control					3744 3632 5216	4197				
2-aminoanthracene w/o S9 positive control*									17 13 17	16
2-aminoanthracene w/ S9 positive control†									416 352 528	432
Methylmethane- Sulfonate w/o S9 positive control									432 416 368	405
Methylmethane- Sulfonate w/ S9 positive control									592 240 1024	619

CFTP = Counts from triplicate plates Mean = Mean of triplicate plates = Not Applicable

*Negative control for S9 †Positive control for S9

Certificate of Quality Assurance Inspections

Phase Inspected	Auditor	Date
Spot Plate Inhibition	K. J. Evener	June 12, 2007
Strain Characteristics	K. J. Evener	June 12, 2007
Final Report Review	J. A. McClure	July 3, 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: Julie A McClure 07-09-07

Julie A. McClure, B.S.
Auditor, Quality Assurance

Date

STORE IN REFRIGERATOR (+4°C)
CALIBRATION #: 7420
TECH/DATE: DmW 5-24-07

GLP SAMPLE SUBMISSI

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T 949 951 3110
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Atlanta, Georgia 30339
T 770 563 1660
F 770 563 1661

o 43619
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F 419 666 2954

07T_36738
25447_001 25447

*Annotates a required field

SPONSOR FINAL REPORT WILL BE ADDRESSED AND MAILED TO

VIDEXX MEDICAL — ATTN* PAUL TIEGE
COMPANY NAME*
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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)*
VC725-186PT
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 Artifical Embolization device
TEST ARTICLE NAME *USE EXACT WORDING DESIRED ON FINAL REPORT* *
Embolotherapy
INTENDED CLINICAL USE OF TEST ARTICLE:*

☑ 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:* 20 vials Occlusin 500
(please specify quantities for each lot/batch/code provided)
glass vials containing white beads
PHYSICAL DESCRIPTION OF TEST ARTICLE (Chemical/Material type/Color)*

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: _____

T052407_015
FEDEX VIREXX
DmW 5-24-07

AUTHORIZED BY SPONSOR — DATE 18 MAY 07
NAMSA STUDY DIRECTOR Michelle E. Longstreth — DATE 5-30-07

REV040207

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:

Genotoxicity: Bacterial Reverse Mutation Study
Soluble - DMSO extraction

TABLE OF CONTENTS

Page

Approvals

Sponsor Representative (Sponsor): _____

Date Approved: 18MAY07

Study Director (NAMSA): Michelle E. Longstreth

Date Initiated: 5-30-07

/esm

1. Introduction

Purpose

The purpose of the study is to evaluate whether an extract of the test material or a solubilized material will cause mutagenic changes in a tryptophan-dependent strain of *Escherichia coli* or in one or more strains of histidine-dependent *Salmonella typhimurium* in the presence or absence of S9 metabolic activation. The Bacterial Reverse Mutation Study will be used as a rapid screening procedure for the determination of mutagenic and potential carcinogenic hazards and should be used in conjunction with other tests that characterize potential genotoxicity properties. This study will be based on OECD guidelines and the requirements of the International Organization for Standardization: Biological Evaluation of Medical Devices - Part 3: Tests for Genotoxicity, Carcinogenicity and Reproductive 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.

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) **Test Article Form:**

2 ✓ Soluble material (solid or liquid) - complete "Preparation of Soluble Material"
___ Insoluble material - complete "Preparation of Extract"
___ Other (specify): _____

Preparation of Extract (for insoluble materials)
Ratio of Test Article to vehicle:

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

Test Article Preparation Instructions:

_____
_____
_____
_____
_____
_____
_____
_____
_____

Extraction Vehicle (select all that apply):

___ 0.9% Sodium Chloride for Injection , USP
MEL 5-31-07 X Dimethyl sulfoxide (DMSO)*
___ 95% ethanol (EtOH)**
___ Other (specify): _____

Extraction Conditions
(use highest temperature that will not degrade material):

X 37°C, 72 hours MEL 5-31-07
___ 50°C, 72 hours
___ 70°C, 24 hours
___ 121°C, 1 hour
___ Room temperature, 72 hours
___ Other (specify): _____

(1) completed by sponsor MEL 5-30-07

*Dimethyl sulfoxide can be extracted at 37° C for 72 hours, 70° C for 24 hours or 50° C for 72 hours.
**95% ethanol can only be extracted at room temperature (various times can be used).

① **Preparation of Soluble Material:**

✓ Solid
One gram of the sample will be transferred to a 10 ml volumetric flask. Various sized flasks may be used to accommodate nature of test material utilizing 100 mg/ml or 10% w/v. Appropriate vehicle (specified below) will be added (q.s.) to the 10 ml (or appropriate) demarcation to achieve 100 mg/ml or a 10% (w/v) solution of the material.

____ Liquid
One milliliter of the sample will be transferred to a 10 ml volumetric flask. Various sized flasks may be used to accommodate nature of test material utilizing 100 mg/ml or 10% v/v. Appropriate vehicle (specified below) will be added (q.s.) to the 10 ml (or appropriate) demarcation to achieve 100 mg/ml or a 10% (v/v) solution of the material.

NOTE: GLP regulations 21 CFR 58.113 requires concentration analysis and stability determination for mixtures with carriers.

① **Vehicles (select all that apply):**

____ 0.9% Sodium Chloride for Injection , USP
✓ Dimethyl sulfoxide (DMSO)*
____ 95% ethanol (EtOH)**
____ Other (specify):_____

All preparations of soluble materials will be performed the day of test. In the event the material does not completely dissolve at these concentrations, serial dilutions will be prepared. The highest possible concentration that achieves complete dissolution of the material will be used for testing purposes.

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

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

① **Special Laboratory Instructions:**

Occlusin 505 has 400 mg of product/vial. Three vials are submitted for this test.

① completed by sponsor MEL 5-30-07

3. Test System

Each *S. typhimurium* tester strain contains a specific mutation in the histidine operon and other mutations that increase their ability to detect mutagens. The *E. coli* strain contains a mutation in the tryptophan operon and a deletion in the *uvrA* gene. These genetically altered *S. typhimurium* strains (TA98, TA100, TA1535, and TA1537) and *E. coli* strain (WP2*uvrA*) cannot grow in the absence of histidine or tryptophan, respectively. When placed in a histidine-free (for *S. typhimurium*) or tryptophan-free (for *E. coli*) medium, only those cells which mutate spontaneously back to their wild type state (histidine independent by manufacturing their own histidine, or tryptophan independent by manufacturing their own tryptophan) are able to form colonies. The spontaneous mutation rate (or reversion rate) for any one strain is relatively constant, but if a mutagen is added to the test system, the mutation rate is significantly increased.

Tester Strain	Mutations/Genotypic Relevance
S. typhimurium TA98	hisD3052, *rfa, uvrB*, frameshift, pKM101
S. typhimurium TA100	hisG46, *rfa*, *uvrB,* missense, pKM101
S. typhimurium TA1535	hisG46, *rfa*, *uvrB*, missense
S. typhimurium TA1537	hisC3076, *rfa*, *uvrB*, frameshift
E. coli WP2*uvrA*	trpE65, *uvrA*, missense

rfa	=	causes partial loss of the lipopolysaccharide wall which increases permeability of the cell to large molecules (i.e., crystal violet inhibition)
uvrB or *uvrA*	=	deficient DNA excision - repair system (i.e., ultraviolet sensitivity)
frameshift	=	base-pair addition/deletion
missense	=	base-pair substitution
pKM101	=	plasmid confers ampicillin resistance (R-factor) and enhances sensitivity to mutagens

Metabolic Activation
Aroclor 1254 - induced rat liver (S9 homogenate) will be used as metabolic activation. The material is prepared from male, Sprague Dawley rats. The rats are induced with one intraperitoneal injection of Aroclor 1254 (500 mg/ml) 5 days prior to sacrifice. The S9 homogenate is purchased from Organon Teknika Corporation, Box 15969, Durham, NC 27704-0969. Just prior to use, the S9 homogenate will be mixed with a buffer containing 0.4 M $MgCl_2$/1.65 M KCl, 1.0 M Glucose-6-phosphate, 0.1 M NADP, 0.2 M sodium phosphate buffer and sterile water.

Preparation of Tester Strains
Cultures of *Salmonella typhimurium*, TA98, TA100, TA1535 and TA1537, and *Escherichia coli*, WP2*uvrA*, will be inoculated to individual Erlenmeyer flasks containing oxoid broth. The inoculated broth cultures will be incubated at 37 ± 2°C in an incubator shaker operating at 115-125 rpm for 10-12 hours.

Preparation of Negative Control
Negative control (vehicle without test material) will be utilized for each tester strain with and without S9 activation.

Preparation of Positive Controls
A known mutagen, Dexon (paradimethylaminobenzene diazosulfonic acid sodium salt), will be used as a positive control to demonstrate that tester strains TA98, TA100, and TA1537 are sensitive to mutation to the wild type state. For tester strain TA1535, sodium azide will be used as a positive control. For tester strain TA100, 2-aminofluorene will be used as a positive control. For tester strain WP2*uvrA*, 2-aminoanthracene and methylmethane-sulfonate will be used as positive controls. Although metabolic activation is only required with 2-aminofluorene and 2-aminoanthracene to induce mutagenic results, all positive controls will be tested with and without S9 homogenate.

Strain Characteristics and Strain Standard Plate Counts
Strain characteristics will be verified and viable counts will be determined.

Spot Plate Inhibition Screen
The extract(s) or solubilized material(s) and negative control(s) will be evaluated by a spot plate technique modeled after the antimicrobial zone of inhibition test. This screen is used to evaluate extract or solution concentrations for toxicity which are noninhibitory to the *Salmonella* strains and the *E. coli* strain.

Separate tubes containing 2 ml of molten top agar supplemented with histidine-biotin (for *S. typhimurium*) or with tryptophan (for *E. coli*) will be inoculated with 0.1 ml of culture for each of the five tester strains. After mixing, the agar will be poured across the surface of separate Minimal E plates labeled with lab number, appropriate tester strain, and dose level (when necessary). Once the agar solidifies, sterile filter discs will be placed in the center of the plates. A 0.1 ml aliquot of the extract

or solubilized material will be added to the filter discs on each of the labeled plates. Parallel testing will be conducted with a negative control. To demonstrate a positive zone of inhibition, 10X stock Dexon will be used.

The plates will be incubated at 37 ± 2°C for 2-3 days. Following the incubation period, the zone of growth inhibition will be recorded. If significant inhibition of the background lawn occurs, the extract or solubilized material concentration will be adjusted by preparing one or more dilutions and repeating the inhibition screen to find a nontoxic level.

4. Methods

Standard Plate Incorporation Assay
Separate tubes containing 2 ml of molten top agar supplemented with histidine-biotin solution (for *S. typhimurium*) or tryptophan (for *E. coli*) will be inoculated with 0.1 ml of culture for each of the five tester strains, and 0.1 ml of the test material. A 0.5 ml aliquot of SWI or S9 homogenate, simulating metabolic activation, will be added when necessary. The mixture will be poured across triplicate Minimal E plates labeled with lab number, appropriate tester strain, and S9 metabolic activation (when applicable). Parallel testing will be conducted on a negative control and five positive controls.

Histidine-free media plates (for *S. typhimurium*) and tryptophan-free media plates (for *E. coli*) will be prepared in triplicate as follows:

1. Extract or solubilized material with and without S9 activation
2. Negative control with and without S9 activation
3. 1X Dexon (known mutagen) with and without S9 activation with strains TA98, TA100, and TA1537
4. 1X 2-aminofluorene (known mutagen) with and without S9 activation with strain TA100
5. 1X Sodium azide (known mutagen) with and without S9 activation with strain TA1535
6. 1X 2-aminoanthracene (known mutagen) with and without S9 activation with strain WP2*uvrA*
7. 1X Methylmethane-sulfonate (known mutagen) with and without S9 activation with strain WP2*uvrA*

The plates will be incubated at 37 ± 2°C for 2-3 days. After the incubation period, the revertant colonies on each plate (test, negative and positive) will be counted and recorded. The mean number of revertants will be calculated. All test methods are described in approved NAMSA Standard Operating Procedures.

5. Evaluation of Test Results

The mean number of revertants of the triplicate test plates will be compared to the mean number of revertants of the triplicate negative control plates for each of the five tester strains employed. The means obtained for the positive controls are used as points of reference.

For a test material to be identified as a test failure or "potential mutagen" there must be a 2-fold or greater increase in the number of mean revertants over the means obtained from the negative control, for any/all five tester strains. If no 2-fold increase is present, the test material is considered nonmutagenic.

Any apparent "positive response" will be confirmed by demonstrating a dose-response relationship using three nontoxic dose levels of the test material. There should be a range of concentrations that produce a linear dose-response. In the event linearity cannot be established, the assay will be repeated with an appropriate change in dose levels. A test material will be judged mutagenic if it causes a dose-related increase in the number of revertants over a minimum of two increasing dose concentrations.

6. Test Validity

For any assay to be considered valid, it must meet the following criteria:

1. Strain characteristics: All *S typhimurium* tester strains (TA98, TA100, TA1535, and TA1537) must exhibit sensitivity to crystal violet (*rfa* mutation), and ultraviolet light (*uvrB*), and must exhibit no growth on biotin plates, and growth on histidine-biotin plates. Tester strains TA98 and TA100 must exhibit resistance to ampicillin (R-factor); tester strains TA1535 and TA1537 must exhibit sensitivity to ampicillin. Tester strain WP2*uvrA* must exhibit sensitivity to ultraviolet light, no growth on tryptophan deficient plates, growth on tryptophan supplemented media and sensitivity to ampicillin.
2. Strain Standard Plate Counts: A viable count on the working culture suspensions for each tester strain (TA98, TA100, TA1535, TA1537 and WP2*uvrA*) should not be less than 1 x 10^8 CFU/ml.
3. Spot Plate Inhibition Screen: Each prepared extract or solubilized material will be evaluated for inhibition or toxicity to the cells. A test sample that is noninhibitory to moderately noninhibitory to the tester strains will be tested by the

standard plate incorporation method. In the event a test material is inhibitory, dilutions will be required to find a nontoxic level.

4. Standard Plate Incorporation Assay: Each positive control mean must exhibit at least a 3-fold increase over the respective negative control mean of the *Salmonella* tester strain employed, and at least a 2-fold increase over the respective negative control mean of the *E. coli* tester strain. Exceptions include conditions not intended to provoke a mutagenic response (e.g. 2-aminoanthracene and 2-aminofluorene without metabolic activation). The negative control results of each tester strain will exhibit a characteristic number of spontaneous revertants. Spontaneous reversion rates may vary, but should be consistent with the ranges specified (see Table II). Table II is meant as a guideline only. Negative control results for tester strains may fall outside of the range listed. In such an instance, the results should be evaluated with caution.

Species	Tester Strain	Number of Spontaneous Revertants
S. typhimurium	TA98	15-50
	TA100	120-240
	TA1537	3-28
	TA1535	10-35
E. coli	WP2*uvrA*	20-125

7. Report

The final report will include all methods used to generate and analyze data. The report will contain the bacterial tester strains employed and characterization including strain standard plate count, spot plate inhibition screen data, test conditions, individual and mean reversion rates (in tabular form), evaluation of results and conclusions.

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 Certificate of Quality Assurance Inspections 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, strain characteristics, standard strain plate counts, spot plate inhibition screen data, standard plate incorporation assay data, and dates of relevant activities 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 for a period of 5 years.

11. References

21 CFR 58 (GLP Regulations).

Ames, B.N., McCann, J., and Yamasaki, E., "Methods for Detecting Carcinogens and Mutagens with the Salmonella/Mammalian-Microsome Mutagenicity Test," Mutation Research 31, (1975): 347-364.

Brusick, D.J., V.F. Simmon, H.S. Rosenkranz, V.A. Ray, and R.S. Stafford, "An Evaluation of the *Escherichia coli* WP2 and WP2*uvrA* Reverse Mutation Assay," Mutation Research 76, (1980): 169-190.

Maron, Dorothy M., Ames, Bruce N., "Revised Methods for the Salmonella Mutagenicity Test," Mutation Research, 113 (1983): 175-215.

International Organization for Standardization 10993-3. Biological Evaluation of Medical Devices, Part 3: Tests for Genotoxicity, Carcinogenicity and Reproductive Toxicity.

OECD Guideline for the Testing of Chemicals, Proposal for Replacement of Guidelines 471 Bacterial Reverse Mutation Test, Document Number 471.

Ortiz, A.I., M.T. Pollastrini, M. Barea, and D. Ordóñez, "Bacterial Mutagenic Evaluation of Luxabendazole, a New Broad Spectrum Antihelminic, with the *Salmonella typhimurium* Histidine and the *Escherichia coli* Tryptophan Reversions Tests," *Mutagenesis* 11 (1996): 27-31.

Test validation, Bacterial Mutagenicity Test: NAMSA lab number 98T-00785-00.

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

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PROTOCOL AMENDMENT I

Test Article: 'Occlusion 500 Artifical Embolization Device

Identification: Batch: FL288

NAMSA Submission ID.: 07T_36738

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:
V0023_211	07T_36738_04	Genotoxicity, Bacterial Reverse Mutation Study - 0.9% SC Extract	June 4, 2007	July 5, 2007
V0023_211	07T_36738_05	Genotoxicity, Bacterial Reverse Mutation Study - DMSO Extract	June 4, 2007	July 5, 2007
T0566_500	07T_36738_06	Mouse Peripheral Blood Micronucleus Study - 0.9% SC Extract	June 4, 2007	August 13, 2007
T0566_501	07T_36738_07	Mouse Peripheral Blood Micronucleus Study - Additional Sample - SO Extract	June 4, 2007	August 13, 2007

Michelle E. Longstreet, B.S.
Study Director

6-4-07
Date

cc: QA (NAMSA)
GLP study file

NAMSA

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PROTOCOL AMENDMENT II

Test Article:	Occlusion 500 Artifical Embolization Device
Identification:	Batch: FL288
Protocol:	V0023_211 Genotoxicity, Bacterial Reverse Mutation Study – 0.9% SC, DMSO Extracts
NAMSA Lab No.:	07T_36738_04, 05
Protocol:	T0566_500 Mouse Peripheral Blood Micronucleus Study – 0.9% SC, SO Extracts
NAMSA Lab No.:	07T_36738_06, 07

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

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PROTOCOL AMENDMENT III

Test Article: Occlusion 500 Artifical Embolization Device

Identification: Batch: FL288

Protocol: V0023_211 Genotoxicity, Bacterial Reverse Mutation Study – DMSO Extract
NAMSA Lab No.: 07T_36738_05

This amendment has been written to correct the **Preparation** section of the study protocols:

- Disregard the extraction section of the protocol. The sample is soluble in DMSO. Use DMSO without extraction as the negative control.

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

6-22-07

Date

cc: QA (NAMSA)
GLP study file