GLP REPORT

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CONFIDENTIAL

STUDY TITLE

Mouse Peripheral Blood Micronucleus Study

TEST ARTICLE NAME

Occlusion 500 Artifical Embolization Device

TEST ARTICLE IDENTIFICATION

Batch: FL288

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Summary

The test article, Occlusion 500 Artifical Embolization Device, Batch: FL288, was extracted in 0.9% sodium chloride USP solution (SC) and sesame oil, NF (SO). The extracts were evaluated for genotoxicity using the Mouse Peripheral Blood Micronucleus model. 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.

For 3 consecutive days (days 1, 2, and 3), twelve mice per test article extract (six per sex) were injected intraperitoneally with the test article extracts. Similarly, twelve mice per extract vehicle were dosed with the appropriate vehicle as the negative control condition and twelve mice were dosed with the positive control, Methyl methanesulfonate. All animals were observed immediately following injection and daily for general health. On day 4, blood was collected from the tail veins and solutions were prepared. The polychromatic erythrocytes were evaluated for the presence of micronuclei. The frequency of micronucleated reticulocytes was determined.

Under the conditions of this study, the test article extracts were not considered to be genotoxic to the mouse. The negative and positive controls performed as expected. There was no evidence of cellular toxicity. 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.

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Study Director

10-16-07
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 — 10-16-07

Michelle E. Longstreet, B.S. — Date

1. Introduction

Purpose
A Mouse Peripheral Blood Micronucleus study was conducted to determine whether a test article extract would cause genotoxic changes in chromosomes or the mitotic apparatus of murine polychromatic erythrocytes. 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. An increase in the frequency of micronucleated reticulocytes (MN-RETs) of treated animals was used as an indication of genetic toxicity.

Dates
The test article was received on May 24, 2007. The animals were initially dosed on September 10, 2007, and sample analysis was concluded on September 24, 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.

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:	Occlusion 500 Artifical Embolization Device
Test Article Identification:	Batch: FL288
Stability Testing:	In progress (per sponsor)
Expiration Date:	Stable for duration of intended testing (per sponsor)
Strength, Purity and Composition:	The sponsor electes not to provide this information to NAMSA and takes full responsibility for this data and can supply this information if requested to do so.
Physical Description of the Test Article:	White beads
Storage Conditions:	Refrigerated
Extraction Vehicles:	0.9% sodium chloride USP solution (SC) Sesame Oil, NF (SO)
Test Article Preparation:	Each vial was filled with the appropriate amount of extract in order to remove the test article from the original container. The test article was prepared based on the surface area of 44 cm^2 per sample. Two samples were included in each preparation. Based on a ratio of 120 cm^2:20 ml, a 88.0 cm^2 portion of the test article was covered with 15 ml of the extract vehicles. The test article was extracted with agitation in the extract vehicles at 37°C for 72 hours. Fresh extracts were prepared for each day of dosing.
Negative Control Articles:	The extraction vehicles, subjected to the same extraction conditions as the test article, were tested to determine the spontaneous occurrence of micronuclei. These data represented a baseline to determine whether the test article had significant clastogenic properties.

Positive Control Article: Methyl methanesulfonate (MMS) in saline, an antineoplastic drug known to have mutagenic properties, was evaluated as the positive control. The positive control was prepared at a concentration of 2.5 mg/ml. Due to the known hazards of this material, safety precautions were followed during the handling of MMS.

Condition of Preparations:

Dose	SC Test Extract	SO Test Extract	SC Negative Control Article	SO Negative Control Article	Positive Control Article
1	Clear with particulates	Clear with particles	Clear	Clear	Clear
2	Clear	Clear with white colored particles	Clear	Clear	Clear
3	Clear with particulates	Clear with white colored particles	Clear	Clear	Clear

3. Test System

Test System

Species: Mouse (*Mus musculus*)
Strain: Hla®:(ICR)CVF®
Source: Hilltop Lab Animals, Inc.
Sex: Thirty male, thirty female
Age: Approximately 7 weeks of age at dosing
Acclimation Period: Minimum 5 days
Number of Animals: Sixty
Identification Method: Ear punch

Justification of Test System

The bone marrow of rodents is routinely used for micronucleus testing since polychromatic erythrocytes are produced in that tissue. The measurement of mirocnucleated immature (polychromatic) erythrocytes in peripheral blood is acceptable in the mouse because these cells have a relatively long lifetime and are not removed by the spleen in the mouse and the species has shown as adequate sensitivity to detect agents that cause structural or numerical chromosome aberration. The intraperitoneal route was selected to maximize delivery of the test article to the target system. Enumeration of micronucleated erythrocytes by traditional microscopic method is a tedious and time-consuming process. Since erythrocytes are devoid of DNA, the cells containing mironuclei can be accurately scored by the image analysis or flow cytometric methods. The MicroFlow method developed by Litron Laboratories is a flow cytometric method that utilizes a single-laser flow cytometer to measure mirocnuclei in the peripheral blood erythrocyte population.

4. Animal Management

Husbandry: Conditions conformed to Standard Operating Procedures that are based on the "Guide for the Care and Use of Laboratory Animals."

Food: A commercially available rodent feed was provided daily.

Water: Potable water was provided *ad libitum* through species appropriate water containers or delivered through an automatic watering system.

Contaminants: Reasonably expected contaminants in feed or water supplies did not have the potential to influence the outcome of this test.

Housing: Animals were housed in groups of six per treatment group and per sex on direct bedding in polycarbonate shoebox cages with filter top lids. Each cage was identified by a card indicating the lab number, animal numbers, test code, sex, treatment group, animal code and date of first dose.

Environment:	The room temperature was monitored daily. The temperature range for the room was within a range of 64-79°F. The room humidity was monitored daily. The humidity range for the room was 30-70%. The light cycle was controlled using an automatic timer (12 hours light, 12 hours dark).
Accreditation:	NAMSA is an AAALAC International accredited facility and is registered with the United States Department of Agriculture. Additionally, NAMSA maintains an approved Animal Welfare Assurance on file with the National Institutes of Health, Office for Laboratory Animal Welfare.
Personnel:	Associates involved were appropriately qualified and trained. The potential hazards of exposure to Methyl methanesulfonate had been thoroughly reviewed by laboratory personnel. All necessary precautions were followed.
Selection:	Only healthy, previously unused animals were selected.
Sedation, Analgesia or Anesthesia:	Sedation, analgesia or anesthesia was not necessary during the routine course of this procedure.
Veterinary Care:	In the unlikely event that an animal became injured, ill, or moribund, care was conducted in accordance with current veterinary medical practice. If warranted for humane reasons, euthanasia was conducted in accordance with the current report of the American Veterinary Medical Association's Panel on Euthanasia. The objective of the study will be given due consideration in any decision and the study sponsor will be advised.
IACUC:	This procedure has been approved by NAMSA Institutional Animal Care and Use Committees (IACUC), and is reviewed at least annually by the same committees. Any significant changes to this procedure were approved by the IACUC prior to conduct.

5. Method

Mice were weighed and distributed into one of the following groups such that the weight variation of animals was minimal and did not exceed ± 20% of the mean weight of each sex.

Group	Treatment	Number of Animals
SC Test	SC Test article extract	6 male, 6 female
SO Test	SO Test article extract	6 male, 6 female
SC Negative Control	0.9% Sodium Chloride	6 male, 6 female
SO Negative Control	Sesame Oil	6 male, 6 female
Positive Control	Methyl methanesulfonate, 50 mg/kg	6 male, 6 female

Each test, negative control, and positive control animal received an intraperitoneal injection of the appropriate article. Injections were administered at a dose of 20 ml/kg. The appropriate dose volume was calculated to the nearest 0.01 ml. The animals were returned to their cages and observed for any adverse reactions immediately after injection. The first day of injections was considered day 1. Animals were similarly injected on day 2 and day 3. The doses were based on the weight of each animal on day 1. Attempts were made to dose the animals at approximately the same time each dosing day.

Animals were observed daily for general health. Body weights were recorded to the nearest whole gram on day 1 and at termination (day 4).

Fixative Tube Preparation
At least one day prior to blood collection, two centrifuge tubes were prepared per mouse from the Mouse MicroFlow^{Basic} Micronucleus analysis kit. Using a pipette, 2 ml of Solution A (fixative) was added to each tube. The tubes were capped and stored at -75 to -80°C at least overnight.

Collection Tube Preparation
Prior to blood collection, one microcentrifuge tube was prepared for each mouse from the Mouse MicroFlow^{Basic} Micronucleus analysis kit. Using a pipette, 350 µl of Solution B (anticoagulant) was added to each tube. The microcentrifuge tubes were stored at 4°C until use. These tubes were moved to room temperature approximately 1 hour before blood collection and remained at room temperature throughout the collection procedure.

Blood Collection/Termination
At 30 ± 6 hours after the last dose, blood was collected from each mouse. Each mouse was placed under a heat lamp for a sufficient time to allow dilation of the tail vein prior to blood collection. The microcentrifuge tube containing anticoagulant for each mouse was shaken immediately prior to collection of the blood. An incision was made in the tail vein of each mouse using a sterile blade. The appropriate microcentrifuge tube was placed under the incision and approximately three drops of blood was collected in the tube. Each tube was capped and inverted several times to mix. Moderate pressure was applied to the incision of each mouse to stop the bleeding. All blood was collected within 36 hours of the last dose. Following the blood collection, the mice were euthanized by carbon dioxide inhalation.

Fixing Blood Samples
The blood/Solution B mixture remained at room temperature for no more than 6 hours before fixing. The centrifuge tubes containing Solution A (fixative) were removed from the ultracold freezer and uncapped two at a time. Approximately 180 µl of the blood/Solution B mixture from each mouse was removed from the microcentrifuge tube and placed in each appropriate centrifuge tube containing fixative. The centrifuge tubes were recapped and mixed. The centrifuge tubes were immediately replaced into the ultracold freezer. The fixed blood samples were stored in the ultracold freezer for a minimum of 24 hours before shipping. After the storage time, five samples/treatment group/sex were randomly selected. These samples were shipped on dry ice to Litron Laboratories. The remaining samples were kept in the ultracold freezer until the sample analysis was complete.

Flow Cytometric Analysis
The flow cytometric analysis was performed at Litron Laboratories. The fixed blood samples were washed and isolated by centrifugation. The blood samples were incubated with RNase to degrade the RNA content of reticulocytes (RETs), a fluorescently labeled antibody to the transferrin receptor (anti-CD71-FITC) to stain the RETs, and a fluorescent labeled antibody that recognizes platelets. Immediately before the analysis, a propidium iodide solution was added to each sample to stain the DNA of the micronuclei. Each blood sample was then analyzed by Flow Cytometric Method (FCM).

6. Evaluation and Statistical Analysis

Up to twenty thousand reticulocytes were analyzed per blood sample. The number of normochromatic erythrocytes (NCEs), micronucleated normochromatic erythrocytes (MN-NCEs), RETs and micronucleated RETs (MN-RETs) were recorded for each sample. The frequency of MN-RETs was determined as an index of genotoxicity. The frequency of reticulocytes relative to total erythrocytes was calculated to provide an indication of stem cell toxicity.

A one-tail t-test was used to determine whether the % MN-RET for the test group was significantly higher than the % MN-RET for the vehicle control group. Calculations resulting in probability (p) values of less than 0.05 were considered statistically significant. However, biological relevance of results was considered in the final determination of genotoxicity.

The test extract was considered non-genotoxic if no statistically significant increase in % MN-RET was observed when compared to the negative control.

The assay was considered valid if the average % MN-RET obtained for the negative control treated animals was between 0.1% and 0.5%. Additionally, the average % MN-RET for the positive control treated animals must be at least 1.0%.

7. Results

Clinical Observations
All animals appeared clinically normal throughout the duration of the study. Weight changes over the course of the study were normal. Individual observations are summarized in Appendix 1.

Sample Analysis
The positive and negative controls performed as anticipated and met the criteria for a valid study. There was no evidence of cellular toxicity. There was no statistically significant increase in the number of MN-RETs for either test extract group. The p-value for the female SO test extract compared to the female SO negative control extract was 0.4019; therefore, there was no statistically significant increase in the number of MN-RETs for the test extract group. The p-value for the female SC test extract compared to the female SC negative control extract was 0.4034; therefore there was no statistically significant increase in the number of MN-RETs for the test extract group. The p-value for the male SC test extract compared to the male SC negative control was 1.000; therefore there was no statistically significant increase in the number of MN-RETs for the test extract group. Statistical evaluation was not performed for the SO test extract for the males as the % MN-RET for this test extract group was not higher than that of the corresponding negative control group. Data from the sample analysis appear in Appendix 2 and 3. Historical performance of the negative and positive controls appears in Appendix 4.

8. Conclusion

Under the conditions of this study, the test article extracts were not considered to be genotoxic to the mouse.

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. Proposed Dates

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

11. Records

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

12. References

21 CFR 58 (GLP Regulations).

Dertinger, S. D., Torous, D. K., and Tometsko, K. T. *Simple and Reliable Enumeration of Micronucleated Reticulocytes with a Single–laser Flow Cytometer*; Mutation Research, 1996: 317, p 283 – 292.

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The Collaborative Study Group for the Micronucleus Test (CSGMT/JEMMS.MMS, The Mammalian Mutagenesis Study Group of the Environmental Mutagen Society of Japan). Protocol Recommended for the Short-term Mouse Peripheral Blood Micronucleus Test. Mutagenesis, 1995: 10, p.153 –159.

Tometsko, A. M., Torous, D. K., and Dertinger, S. D. *Analysis of Micronucleated Cells by Flow Cytometry. 3. Advanced Technology for Detecting Clastogenic Activity*; Mutation Research, 1993: 292, p. 145-153.

Tometsko, A. M., Dertinger, S. D., and Torous, D. K. Analysis of Micronucleated Cells by Flow Cytometry. 4. Kinetic Analysis of Cytogenic Damage in Blood; Mutation Research, 1995: 334, p. 9-18.

Torous, D. K, et al. *An Automated Method for Discriminating Aneugen vs. Clastogen-induced Micronuclei*; Environmental and Molecular Mutagenesis, 1998: 31, p. 340-344.

Torous, D. K. et al. *Flow Cytometric Enumeration of Micronucleated Reticulocytes: High Transferability Among 14 Laboratories*; Environmental and Molecular Mutagenesis, 2001: p. 59-68.

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

13. Protocol Changes

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

Appendix 1 - Individual Body Weight and Health Observations

(Positive Control)

Treatment Group	Gender	Animal Number	Body Weights (g)		Health Observations			
			Day 1	Day 4	Day 1	Day 2	Day 3	Day 4
Positive Control	Male	1	30	31	AN	AN	AN	AN
		2	31	31	AN	AN	AN	AN
		3	30	29	AN	AN	AN	AN
		4	27	28	AN	AN	AN	AN
		5	30	30	AN	AN	AN	AN
		6	29	30	AN	AN	AN	AN
		Mean:	30					
	Female	7	23	23	AN	AN	AN	AN
		8	24	25	AN	AN	AN	AN
		9	24	24	AN	AN	AN	AN
		10	23	23	AN	AN	AN	AN
		11	23	23	AN	AN	AN	AN
		12	23	23	AN	AN	AN	AN
		Mean:	23					

AN = Appeared Normal

Appendix 1 (continued) - Individual Body Weight and Health Observations

(SC)

Treatment Group	Gender	Animal Number	Body Weights (g)		Health Observations			
			Day 1	Day 4	Day 1	Day 2	Day 3	Day 4
Test	Male	37	29	30	AN	AN	AN	AN
		38	30	30	AN	AN	AN	AN
		39	28	28	AN	AN	AN	AN
		40	30	30	AN	AN	AN	AN
		41	29	30	AN	AN	AN	AN
		42	30	31	AN	AN	AN	AN
		Mean:	29					
	Female	43	22	23	AN	AN	AN	AN
		44	23	23	AN	AN	AN	AN
		45	23	24	AN	AN	AN	AN
		46	22	23	AN	AN	AN	AN
		47	23	23	AN	AN	AN	AN
		48	22	22	AN	AN	AN	AN
		Mean:	23					
Negative Control	Male	13	29	29	AN	AN	AN	AN
		14	27	27	AN	AN	AN	AN
		15	29	29	AN	AN	AN	AN
		16	30	31	AN	AN	AN	AN
		17	27	28	AN	AN	AN	AN
		18	29	29	AN	AN	AN	AN
		Mean:	29					
	Female	19	23	23	AN	AN	AN	AN
		20	23	23	AN	AN	AN	AN
		21	22	23	AN	AN	AN	AN
		22	22	21	AN	AN	AN	AN
		23	23	24	AN	AN	AN	AN
		24	23	24	AN	AN	AN	AN
		Mean:	23					

AN = Appeared Normal

Appendix 1 (continued) - Individual Body Weight and Health Observations

(SO)

Treatment Group	Gender	Animal Number	Body Weights (g)		Health Observations			
			Day 1	Day 4	Day 1	Day 2	Day 3	Day 4
Test	Male	49	29	30	AN	AN	AN	AN
		50	28	29	AN	AN	AN	AN
		51	32	32	AN	AN	AN	AN
		52	27	28	AN	AN	AN	AN
		53	30	33	AN	AN	AN	AN
		54	27	28	AN	AN	AN	AN
		Mean:	29					
	Female	55	23	23	AN	AN	AN	AN
		56	23	24	AN	AN	AN	AN
		57	23	25	AN	AN	AN	AN
		58	23	23	AN	AN	AN	AN
		59	23	23	AN	AN	AN	AN
		60	24	24	AN	AN	AN	AN
		Mean:	23					
Negative Control	Male	25	27	29	AN	AN	AN	AN
		26	26	28	AN	AN	AN	AN
		27	26	29	AN	AN	AN	AN
		28	28	30	AN	AN	AN	AN
		29	30	32	AN	AN	AN	AN
		30	28	29	AN	AN	AN	AN
		Mean:	28					
	Female	31	21	23	AN	AN	AN	AN
		32	23	23	AN	AN	AN	AN
		33	22	23	AN	AN	AN	AN
		34	21	22	AN	AN	AN	AN
		35	21	23	AN	AN	AN	AN
		36	23	24	AN	AN	AN	AN
		Mean:	22					

AN = Appeared Normal

Appendix 2 - Calculated Frequencies Evaluation Data

(Positive Control)

Treatment Group	Gender	Animal Number	% RET	% MN-NCE	% MN-RET
Positive Control	Male	1	2.81	0.26	3.08
		2	1.97	0.34	4.32
		3	1.24	0.31	5.51
		5	0.54	0.31	7.81
		6	1.15	0.30	4.58
	Average		1.54	0.30	5.06
	Standard Deviation		0.87	0.03	1.77
	Female	7	1.84	0.16	2.17
		8	0.98	0.20	6.75
		9	1.10	0.26	4.54
		10	1.59	0.14	1.32
		11	1.85	0.16	2.47
	Average		1.47	0.18	3.45
	Standard Deviation		0.41	0.05	2.19

% RET = frequency (%) of CD71 positive reticulocytes
% MN-NCE = frequency (%) of micronucleated normochromatic erythrocytes
% MN-RET = frequency (%) of CD71 positive micronucleated reticulocytes

Appendix 2 (continued) - Calculated Frequencies Evaluation Data

(SC)

Treatment Group	Gender	Animal Number	% RET	% MN-NCE	% MN-RET
Test	Male	37	3.57	0.22	0.29
		38	2.59	0.21	0.31
		39	2.77	0.18	0.29
		40	2.63	0.27	0.41
		42	3.32	0.20	0.28
	Average		2.98	0.22	0.32
	Standard Deviation		0.44	0.03	0.05
	Female	43	1.34	0.13	0.34
		44	2.25	0.14	0.22
		45	3.93	0.17	0.27
		47	3.03	0.19	0.24
		48	5.30	0.14	0.35
	Average		3.17	0.15	0.28
	Standard Deviation		1.53	0.03	0.06
Negative Control	Male	13	3.12	0.21	0.32
		14	3.04	0.22	0.31
		15	2.97	0.18	0.28
		17	2.33	0.26	0.30
		18	2.45	0.21	0.29
	Average		2.78	0.22	0.30
	Standard Deviation		0.36	0.03	0.02
	Female	19	2.46	0.11	0.18
		20	1.96	0.15	0.28
		21	2.82	0.15	0.30
		23	1.48	0.11	0.14
		24	1.85	0.12	0.25
	Average		2.11	0.13	0.23
	Standard Deviation		0.53	0.02	0.07

% RET = frequency (%) of CD71 positive reticulocytes
% MN-NCE = frequency (%) of micronucleated normochromatic erythrocytes
% MN-RET = frequency (%) of CD71 positive micronucleated reticulocytes

Appendix 2 (continued) - Calculated Frequencies Evaluation Data

(SO)

Treatment Group	Gender	Animal Number	% RET	% MN-NCE	% MN-RET
Test	Male	49	2.68	0.22	0.36
		50	5.17	0.20	0.35
		51	3.20	0.24	0.36
		53	1.90	0.22	0.31
		54	2.30	0.16	0.19
	Average		3.05	0.21	0.31
	Standard Deviation		1.28	0.03	0.07
	Female	55	2.43	0.13	0.23
		56	3.77	0.18	0.36
		57	2.38	0.14	0.30
		59	1.86	0.14	0.16
		60	1.97	0.17	0.31
	Average		2.48	0.15	0.27
	Standard Deviation		0.76	0.02	0.08
Negative Control	Male	26	2.06	0.24	0.29
		27	3.40	0.16	0.30
		28	2.28	0.20	0.31
		29	2.80	0.21	0.39
		30	3.37	0.16	0.25
	Average		2.78	0.19	0.31
	Standard Deviation		0.61	0.03	0.05
	Female	32	2.35	0.15	0.30
		33	3.78	0.14	0.26
		34	2.85	0.15	0.17
		35	2.83	0.17	0.35
		36	1.86	0.17	0.22
	Average		2.73	0.16	0.26
	Standard Deviation		0.71	0.01	0.07

% RET = frequency (%) of CD71 positive reticulocytes
% MN-NCE = frequency (%) of micronucleated normochromatic erythrocytes
% MN-RET = frequency (%) of CD71 positive micronucleated reticulocytes

(Positive Control)

Treatment Group	Gender	Animal Number	Number of NCE	Number of MN-NCE	Number of RET	Number of MN-RET
Positive Control	Male	1	688896	1763	19385	615
		2	989758	3375	19136	864
		3	1586102	4922	18898	1102
		5	3676049	11404	18439	1561
		6	1713662	5161	19085	915
	Female	7	1064623	1735	19566	434
		8	2007595	4078	18650	1350
		9	1794219	4659	19093	907
		10	1237319	1790	19736	264
		11	1060466	1705	19507	493

NCE = normochromatic erythrocytes
MN-NCE = micronucleated normochromatic erythrocytes
RET = CD71 positive reticulocytes
MN-RET = CD71 positive micronucleated reticulocytes

(SC)

Treatment Group	Gender	Animal Number	Number of NCE	Number of MN-NCE	Number of RET	Number of MN-RET
Test	Male	37	539502	1190	19942	58
		38	750142	1570	19938	62
		39	701838	1263	19942	58
		40	737855	1982	19918	82
		42	581979	1180	19944	56
	Female	43	1465803	1897	19933	67
		44	866663	1173	19956	44
		45	487959	808	19947	53
		47	639263	1186	19952	48
		48	357019	501	19931	69
Negative Control	Male	13	618771	1281	19936	64
		14	637487	1391	19939	61
		15	653162	1174	19945	55
		17	836440	2143	19941	59
		18	795972	1709	19942	58
	Female	19	790920	908	19965	35
		20	1001195	1498	19945	55
		21	687803	1030	19940	60
		23	1333003	1517	19973	27
		24	1059494	1325	19951	49

NCE = normochromatic erythrocytes
MN-NCE = micronucleated normochromatic erythrocytes
RET = CD71 positive reticulocytes
MN-RET = CD71 positive micronucleated reticulocytes

(SO)

Treatment Group	Gender	Animal Number	Number of NCE	Number of MN-NCE	Number of RET	Number of MN-RET
Test	Male	49	724871	1614	19928	72
		50	366353	740	19930	70
		51	603444	1467	19929	71
		53	1031740	2257	19938	62
		54	846559	1352	19962	38
	Female	55	802792	1039	19955	45
		56	509242	929	19928	72
		57	818347	1123	19941	59
		59	1054430	1501	19969	31
		60	993430	1710	19938	62
Negative Control	Male	26	947018	2239	19942	58
		27	568214	886	19941	59
		28	856582	1730	19938	62
		29	693269	1489	19922	78
		30	572451	924	19951	49
	Female	32	831454	1260	19941	59
		33	508152	714	19949	51
		34	680519	1030	19966	34
		35	686814	1147	19930	70
		36	1053959	1771	19957	43

NCE = normochromatic erythrocytes
MN-NCE = micronucleated normochromatic erythrocytes
RET = CD71 positive reticulocytes
MN-RET = CD71 positive micronucleated reticulocytes

Appendix 4 - Historical Negative And Positive Control Data*

Table 1: Negative Control (Saline)				
	% RET		% MN-RET	
Male	Mean	2.52	Mean	0.19
	SD	0.54	SD	0.05
	Range	0.97 – 5.51	Range	0.07-0.47
Female	Mean	2.27	Mean	0.19
	SD	0.56	SD	0.05
	Range	0.56-4.79	Range	0.07-0.42

Table 2: Negative Control (Sesame Oil)				
	% RET		% MN-RET	
Male	Mean	2.53	Mean	0.18
	SD	0.57	SD	0.04
	Range	0.89-9.32	Range	0.05-0.43
Female	Mean	2.51	Mean	0.18
	SD	0.61	SD	0.04
	Range	0.76-5.65	Range	0.06-0.43

Table 3: Positive Control (MMS)				
	% RET		% MN-RET	
Male	Mean	1.41	Mean	3.24
	SD	0.50	SD	1.05
	Range	0.23-7.16	Range	0.50-8.62
Female	Mean	1.72	Mean	2.33
	SD	0.55	SD	0.77
	Range	0.33-4.09	Range	0.38-7.22

*Based on assays performed by NAMSA.

Statement of Quality Assurance Activities

Phase Inspected	Auditor	Date
Termination	R. J. Spino	September 13, 2007
Study Data Approval	H. A. Saums	September 28, 2007
Study Data Approval	H. A. Saums	October 3, 2007
Final Report Review	R. J. Spino	October 16, 2007

Reports to Management and Study Director(s)	Date
Periodic Status Report	June 8, 2007
Periodic Status Report	July 10, 2007
Periodic Status Report	August 10, 2007
Periodic Status Report	September 10, 2007
Periodic Status Report	October 10, 2007

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

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

QA Representative: _____ 10-16-07

Ryan J. Spino, B.S.
Auditor, Quality Assurance

Date

STORE IN REFRIGERATOR (+4°C)
CALIBRATION #: 7420
TECH/DATE: DMW 5.24-07

GLP SAMPLE SUBMISSI

07T_36738
25447_001 25447

USA Corporate Headquarters
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*Annotates a required field

SPONSOR FINAL REPORT WILL BE ADDRESSED AND MAILED TO

COMPANY NAME*: VIREXX MEDICAL ATTN*: PAUL TIEGE
ADDRESS*: 8223 Roper Rd
CITY*: Edmonton STATE*: AB 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)*: same, attn Erin Horwitz
PURCHASE ORDER NUMBER*: VC725-186PT
COST ESTIMATE AND PROPOSAL NUMBER: T07 2708
☐ VISA ☐ MasterCard ☐ American Exp.
CARD HOLDER NAME
CREDIT CARD NUMBER EXPIRATION DATE
ACCOUNTS PAYABLE PHONE* ACCOUNTS PAYABLE FAX*

TEST ARTICLE NAME *USE EXACT WORDING DESIRED ON FINAL REPORT**: Occlusin 500 Artificial Embolization Device
INTENDED CLINICAL USE OF TEST ARTICLE:*: Embolotherapy

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

CONTROL ARTICLE NAME*:
CHECK ONE: ☐ BATCH ☐ CODE ☐ LOT 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)
PHYSICAL DESCRIPTION OF TEST ARTICLE (Chemical/Material type/Color)*: glass vials containing white beads

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

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

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

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

STORAGE CONDITIONS*
☐ ROOM TEMPERATURE ☑ 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: Paul Tiege DATE: 18 MAY 07
NAMSA STUDY DIRECTOR: Michelle E. Longstreet DATE: 5-30-07

REV040207

TEST FACILITY:

NAMSA
6750 Wales Road
Northwood, OH 43619-1011

SPONSOR:

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

STUDY TITLE:

Mouse Peripheral Blood Micronucleus Study

TABLE OF CONTENTS Page

Approvals

Sponsor Representative (Sponsor): _____

Date Approved: 18 MAY 07

Study Director (NAMSA): Michelle E. Longstaff

Date Initiated: 5-30-07

1. Introduction

Purpose
The objective of this *in vivo* procedure is to evaluate the potential of an extract of the test article to cause genotoxic changes in the chromosomes or the mitotic apparatus of murine polychromatic erythrocytes. Genetic toxicity is indicated by an increase in the frequency of micronucleated reticulocytes (MN-RET) of treated animals. The test also utilizes the frequency of reticulocytes relative to total erythrocytes to provide an indicator of stem cell toxicity. This study is based on the requirements of the International Organization for Standardization (ISO): Biological evaluation of medical devices - Part 3: Tests for genotoxicity, carcinogenicity, and reproductive toxicity, the Organisation for Economic Co-operation and Development (OECD) Test No. 474: Mammalian Erythrocyte Micronucleus Test, and the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) Tripartite Harmonised Guideline SA2: Guidance on Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals.

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

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

2. Materials

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

Preparation
The following is to be completed by the sponsor or study director. Further instructions may be attached to the protocol. A fresh extract will be prepared for each dosing interval. The sample will be prepared as follows:

Insoluble test article

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

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

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

each vial of exclusion 505 has a total SA per vial of 44 cm^2
please extract the contents of 3 vials, 132 cm^2, in an appropriate volume for each extract. (3 vials saline and 3 vials oil)

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

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

- ✓ 0.9% sodium chloride USP solution (saline)
- ✓ ~~Vegetable oil~~ sesame oil MEL 5-30-07

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

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

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

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

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

Special Laboratory Instructions:

Control Article

Negative Control: The extraction vehicle will serve as the negative control.

Positive Control: The test facility will provide Methyl methanesulfonate (MMS), an antineoplastic drug known to have mutagenic properties. The positive control will be prepared in saline at a concentration of 2.5 mg/ml. Due to the known hazards of this material, safety precautions will be taken while handling this compound. Disposable gloves, safety glasses and designated laboratory clothing will be worn at all times. All materials that come in contact with MMS will be disposed of according to approved procedures.

3. Test System

Test System

Species:	Mouse (*Mus musculus*)
Strain:	Outbred albino
Source:	NAMSA approved supplier
Sex:	Male and female
Age:	6 to 8 weeks of age at dosing
Acclimation Period:	Minimum 5 days
Number of Animals:	Six male and six female per each extract and control
Identification Method:	Ear punch

12 saline 12 saline

72 mice!

12 saline test 12 eve control 12 ⊕ control

12 oil test 12 eve control 12 ⊕ control

Justification of Test System

The bone marrow of rodents is routinely used for micronucleus testing since polychromatic erythrocytes are produced in that tissue. The measurement of micronucleated immature (polychromatic) erythrocytes in peripheral blood is acceptable in the mouse because these cells have a relatively long lifetime and are not removed by the spleen in the mouse and the species has shown as adequate sensitivity to detect agents that cause structural or numerical chromosome aberration. The intraperitoneal route was selected to maximize delivery of the test article to the target system. Enumeration of micronucleated erythrocytes by traditional microscopic method is a tedious and time-consuming process. Since erythrocytes are devoid of DNA, the cells containing micronuclei can be accurately scored by the image analysis or flow cytometric methods. The MicroFlow method developed by Litron Laboratories is a flow cytometric method that utilizes a single-laser flow cytometer to measure micronuclei in the peripheral blood erythrocyte population.

4. Animal Management

Husbandry:	Conditions will conform to Standard Operating Procedures that are based on the "Guide for the Care and Use of Laboratory Animals."
Food:	A commercially available rodent feed will be provided daily.
Water:	Potable water will be provided *ad libitum* through species appropriate water containers or delivered through an automatic watering system.
Contaminants:	Reasonably expected contaminants in feed or water supplies should not have the potential to influence the outcome of this test.
Housing:	Animals will be housed in groups of six per treatment group per sex on direct bedding in polycarbonate shoebox cages with filter top lids. Each cage will be identified by a card indicating the lab number, animal numbers, test code, sex, treatment group, animal code and date of first dose.
Environment:	The room temperature will be monitored daily. The recommended temperature range for the room is 64-79°F. The room humidity will be monitored daily. The humidity range for the room is 30-70%.

The light cycle will be controlled using an automatic timer (12 hours light, 12 hours dark).

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. The potential hazards of exposure to Methyl methanesulfonate will have been thoroughly reviewed by laboratory personnel. All necessary precautions will be followed.

Selection: Only healthy, previously unused animals will be selected.

Sedation, Analgesia or Anesthesia: It has been determined that the use of sedation, analgesia or anesthesia will not be necessary during the routine course of this procedure.

Veterinary Care: In the unlikely event that an animal should become injured, ill, or moribund, care will be conducted in accordance with current veterinary medical practice. If warranted for humane reasons, euthanasia will be conducted in accordance with the current report of the American Veterinary Medical Association's Panel on Euthanasia. The objective of the study will be given due consideration in any decision and the study sponsor will be advised.

IACUC: This protocol has been approved by NAMSA Institutional Animal Care and Use Committees (IACUC), and is reviewed at least annually by the same committees. Any significant changes to this protocol must be approved by the IACUC prior to conduct.

5. Method

Mice will be weighed and distributed into one of the following groups such that the weigh variation is minimal and does not exceed ± 20% of the mean weight of each sex.

Group	Treatment	Number of Animals
Test	Test article extract	6 male, 6 female
Negative Control	Vehicle	6 male, 6 female
Positive Control	Methyl methanesulfonate, 50 mg/kg	6 male, 6 female

Each test, negative control, and positive control animal will receive an intraperitoneal injection of the appropriate article. Injections will be administered at a dose of 20 ml/kg. The appropriate dose volume will be calculated to the nearest 0.01 ml. The animals will be returned to their cages. The first day of injections will be considered day 1. Animals will be similarly injected on day 2 and day 3. The doses will be based on the weight of each animal on day 1. Attempts will be made to dose the animals at approximately the same time each dosing day.

Laboratory Observations

1. Animals will be observed daily for general health. Mice will also be observed for any adverse reactions immediately after injection.
2. Body weights will be recorded to the nearest whole gram on day 1 and at termination (day 4).
3. A general necropsy will be conducted on any animal that dies or exhibits adverse clinical signs that necessitates euthanasia prior to the termination date. Animals will not be replaced.

Fixative Tube Preparation

At least one day prior to blood collection, two centrifuge tubes will be prepared per mouse from the Mouse MicroFlow^{Basic} Micronucleus analysis kit. Using a pipette, 2 ml of Solution A (fixative) will be added to each tube. The tubes will be capped and stored at -75 to -80°C at least overnight.

Collection Tube Preparation

Prior to blood collection, one microcentrifuge tube will be prepared for each mouse from the Mouse MicroFlow^{Basic} Micronucleus analysis kit. Using a pipette, 350 µl of Solution B (anticoagulant) will be added to each tube. The microcentrifuge tubes will be stored at 4°C until use. These tubes will be moved to room temperature approximately 1 hour before blood collection and will remain at room temperature throughout the collection procedure.

Blood Collection/Termination
At 30 ± 6 hours after the last dose, blood will be collected from each mouse. Each mouse will be placed under a heat lamp for a sufficient time to allow dilation of the tail vein prior to blood collection. The microcentrifuge tube containing anticoagulant for each mouse will be shaken immediately prior to collection of the blood. An incision will be made in the tail vein of each mouse using a sterile blade. The appropriate microcentrifuge tube will be placed under the incision and approximately three drops of blood will be collected in the tube. Each tube will be capped and inverted several times to mix. Moderate pressure will be applied to the incision of each mouse to stop the bleeding. All blood must be collected within 36 hours after the last dose. Following the blood collection, the mice will be euthanized by carbon dioxide inhalation.

Fixing Blood Samples
The blood/Solution B mixture can remain at room temperature for up to 6 hours before fixing. The centrifuge tubes containing Solution A (fixative) will be removed from the ultracold freezer and uncapped two at a time. Approximately 180 µl of the blood/Solution B mixture from each mouse will be removed from the microcentrifuge tube and placed in each appropriate centrifuge tube containing fixative. The centrifuge tubes will be recapped and mixed. The centrifuge tubes will be immediately replaced into the ultracold freezer. The fixed blood samples will be stored in the ultracold freezer for a minimum of 24 hours before shipping. After the storage time, randomly select five samples/treatment group/sex. These samples will be shipped on dry ice to Litron Laboratories. The remaining samples will be kept in the ultracold freezer until the sample analysis is complete.

Flow Cytometric Analysis
The flow cytometric analysis will be performed at Litron Laboratories. The fixed blood samples will be washed and isolated by centrifugation. The blood samples will be incubated with RNase to degrade the RNA content of reticulocytes (RETs), a fluorescently labeled antibody to the transferrin receptor (anti-CD71-FITC) to stain the RETs, and a fluorescent labeled antibody that recognizes platelets. Immediately before the analysis, a propidium iodide solution will be added to each sample to stain the DNA of the micronuclei. Each blood sample will then be analyzed by Flow Cytometric Method (FCM).

6. Evaluation and Statistical Analysis

Up to twenty thousand reticulocytes will be analyzed per blood sample. The number of normochromatic erythrocytes (NCEs), micronucleated normochromatic erythrocytes (MN-NCEs), RETs and micronucleated RETs (MN-RETs) will be recorded for each sample. The frequency of MN-RETs will be determined as an index of genotoxicity. The frequency of reticulocytes relative to total erythrocytes will be calculated to provide an indication of stem cell toxicity.

A one-tail t-test will be used to determine whether the % MN-RET for the test group is significantly higher than the % MN-RET for the vehicle control group. Calculations resulting in probability (p) values of less than 0.05 will be considered statistically significant. However, biological relevance of results will be considered in the final determination of genotoxicity.

7. Acceptance Criteria

The study will be considered valid if the following criteria are met:

- The average %MN-RET for the vehicle control group must be between 0.1% and 0.5%
- The average %MN-RET for the positive control group (MMS) must be at least 1.0%

8. Report

The final report will include a description of the test article preparation, the methods employed, the proportion of immature erythrocytes among total erythrocytes, the number of micronucleated immature erythrocytes among total immature erythrocytes for each animal presented in tabular form, if appropriate and applicable, the number of micronucleated mature erythrocytes among total mature erythrocytes for each animal presented in tabular form, mean ± standard deviation of micronucleated immature erythrocytes per group, clinical observations, body weight data, a summary of results, historical negative and positive control data, statistical data, and conclusions.

9. Quality Assurance

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

10. Records

Test article preparation, counting 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.

11. 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) and added to the protocol.

12. References

21 CFR 58 (GLP Regulations).

Dertinger, S. D., Torous, D. K., and Tometsko, K. T. *Simple and Reliable Enumeration of Micronucleated Reticulocytes with a Single–laser Flow Cytometer*; Mutation Research, 1996: 317, p 283 – 292.

Dertinger, S. D., Torous, D. K., and Tometsko, K. T. *Flow Cytometric Analysis of Micronucleated Reticulocytes in Mouse Bone Marrow*; Mutation Research, 1997: 390, p. 257 – 262.

FDA Redbook (2000), *Mammalian Erythrocyte Micronucleus Test*; section IV.C.1.d.

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

Hayashi, M., Tice, R. R. et al. *In vivo Rodent Micronucleus Assay*; Mutation Research, 1994: 312, p. 293 – 304.

Hayashi, M. et al. *In vivo Rodent Erythrocyte Micronucleus Assay II*; Environmental and Molecular Mutagenesis, 2000: 35, p. 234 – 252.

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

Mavourin, K. H., Blakey, D. H. et al. *The in vivo Micronucleus Assay in Mammalian Bone Marrow and Peripheral Blood*, A Report of the U.S. Environmental Protection Agency Gene-Tox Program. Mutation Research, 1990: 239, p. 29-80.

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

OECD Test No. 474: Mammalian Erythrocyte Micronucleus Test (1997).

The Collaborative Study Group for the Micronucleus Test. Micronucleus Test with Mouse Peripheral Blood Erythrocytes by Acridine Orange Supravital Staining: The Summary Report of the 5th Collaborative Study by CSGMT/JEMS.MMS. Mutation Research, 1992: 278, p. 83 – 98.

The Collaborative Study Group for the Micronucleus Test (CSGMT/JEMMS.MMS, The Mammalian Mutagenesis Study Group of the Environmental Mutagen Society of Japan). Protocol Recommended for the Short-term Mouse Peripheral Blood Micronucleus Test. Mutagenesis, 1995: 10, p.153 –159.

Tometsko, A. M., Torous, D. K., and Dertinger, S. D. *Analysis of Micronucleated Cells by Flow Cytometry. 3. Advanced Technology for Detecting Clastogenic Activity*; Mutation Research, 1993: 292, p. 145-153.

Tometsko, A. M., Dertinger, S. D., and Torous, D. K. Analysis of Micronucleated Cells by Flow Cytometry. 4. Kinetic Analysis of Cytogenic Damage in Blood; Mutation Research, 1995: 334, p. 9-18.

Torous, D. K, et al. *An Automated Method for Discriminating Aneugen vs. Clastogen-induced Micronuclei*; Environmental and Molecular Mutagenesis, 1998: 31, p. 340-344.

Torous, D. K. et al. *Flow Cytometric Enumeration of Micronucleated Reticulocytes: High Transferability Among 14 Laboratories*; Environmental and Molecular Mutagenesis, 2001: p. 59-68.

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

13. Protocol Changes

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

NAMSA

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June 4, 2007

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

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

Michelle E. Longstreet, B.S.
Study Director

6-4-07
Date

cc: QA (NAMSA)
GLP study file

NAMSA

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October 11, 2007

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

REVISED*
PROTOCOL AMENDMENT I

Test Article: Occlusion 500 Artifical Embolization Device

Identification: Batch: FL288

NAMSA Submission ID.: 07T_36738

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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	October 27, 2007*
T0566_501	07T_36738_07	Mouse Peripheral Blood Micronucleus Study - Additional Sample - SO Extract	June 4, 2007	October 27, 2007*

*This amendment has been revised to correct the estimated report release dates.

Michelle E. Longstreet

Michelle E. Longstreet, B.S.
Study Director

10-11-07
Date

cc: QA (NAMSA)
GLP study file

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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)
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PROTOCOL AMENDMENT III

Test Article: Occlusion 500 Artifical Embolization Device

Identification: Batch: FL288

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:

- Use 6 vials for preparation of each extract. Each vial has a surface area of 44 cm^2.

This amendment to the protocol was written prior to testing.

Michelle E. Longstreet, B.S.
Study Director

8-14-07
Date

cc: QA (NAMSA)
GLP study file