TEST FACILITY

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SPONSOR

Geoff Daly
Analytica Ltd
85 Brandl Street, Eight Mile Plains
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STUDY TITLE

ASTM Hemolysis

TEST ARTICLE NAME

Analytica AutoStart 150mL Burette

TEST ARTICLE IDENTIFICATION

Lot: 20080909

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Summary

The test article, Analytica AutoStart 150mL Burette, Lot: 20080909, was evaluated based on ASTM F756, Standard Practice for Assessment of Hemolytic Properties of Materials and also per the requirements of ISO 10993-4, Biological Evaluation of Medical Devices – Part 4: Selection of Tests for Interactions With Blood. Blood was obtained from three rabbits, pooled, and diluted for use in this study. Diluted rabbit blood was added to triplicate tubes of the test article in calcium and magnesium-free phosphate buffered saline (CMF-PBS) and triplicate tubes of the CMF-PBS test article extract. These combinations were evaluated to determine whether direct contact with the test article or an extract of the test article would cause *in vitro* red blood cell hemolysis. Negative and positive controls were prepared in the same manner as the test article. Each tube was inverted gently to uniformly mix the contents with the blood. The tubes were then maintained for 3 hours at 37°C with periodic inversions. Following incubation, suspensions were mixed gently and centrifuged. The resulting supernatant was added to hemoglobin reagent. The absorbances of the solutions were spectrophotometrically measured at a wavelength of 540 nm.

Under the conditions of this study, the mean hemolytic index for the test article in CMF-PBS was 0%, and the mean hemolytic index for the CMF-PBS test article extract was 0%. The direct contact of the test article was nonhemolytic and the test article extract was nonhemolytic. The negative and positive controls performed as anticipated.

Study and Supervisory Personnel:

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Approved by:

Jolee Bartrom 10-20-08

Jolee Bartrom, B.S.
Study Director

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: Jolee Bartrom 10-20-08

Jolee Bartrom, B.S. Date

1. Introduction

Purpose
The test article identified below was evaluated to determine whether the test article would cause hemolysis *in vitro* by direct contact or extraction per ASTM F756, Standard Practice for Assessment of Hemolytic Properties of Materials and also per the requirements of ISO 10993-4, Biological Evaluation of Medical Devices - Part 4: Selection of Tests for Interactions With Blood. Hemolysis testing of medical device materials has been used historically to measure blood compatibility.

Dates
The test article was received on September 15, 2008. The test was performed on October 9, 2008.

GLP Compliance
The study initiated by protocol signature on September 15, 2008, was conducted in accordance with the provisions of the FDA Good Laboratory Practice (GLP) Regulations, 21 CFR 58. A Statement of Quality Assurance Activities was issued with this report.

2. Materials

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

Test Article Name:	Analytica AutoStart 150mL Burette
Test Article Identification:	Lot: 20080909
Stability Testing:	In progress (per sponsor)
Expiration Date:	Stable for duration of intended testing (per sponsor)
Strength, Purity and Composition:	Strength: Not applicable as there are no active ingredients are used to formulate a concentration; Purity: Not applicable, because the test article is a multi-component device with no active ingredients; Composition: See Appendix 1.
Physical Description of the Test Article:	Single-use, sterile, medical device. Predominantly transparent PVC and white ABS
Storage Conditions:	Room Temperature
Vehicle:	Calcium and magnesium-free phosphate buffered saline (CMF-PBS)
Test Article Preparation:	One device was filled with a total of 131.2 ml of the vehicle to be tested as the direct contact. The device was sealed as necessary to avoid loss of the vehicle during extraction. One device was filled with a total of 159 ml of the vehicle. The device was sealed as necessary to avoid loss of the vehicle during extraction. These preparations were extracted with agitation at 50°C for 72 hours.
Negative Control Preparation:	High density polyethylene (HDPE) was used as the negative control. Based on the USP ratio of 60 cm^2:20 ml, triplicate 21.0 cm^2 portions of HDPE were prepared and covered with 7.0 ml of CMF-PBS to be tested as direct contact. Based on the USP ratio of 60 cm^2:20 ml, triplicate 30.8 cm^2 portions of HDPE were prepared and covered with 10 ml of CMF-PBS. These preparations were subjected to the extraction conditions previously described for the test article.
Stability Testing:	Marketed product stability characterized by its labeling

Strength, Purity and Composition:	Strength: Not applicable, no active components in the formulation. Purity: Meets USP <661> polyethylene containers, multiple internal reflectance, thermal analysis, heavy metals, and non-volatile residue. Composition: Neat CAS #: 9002-88-4
Positive Control Preparation:	Sterile Water for Injection (SWFI) was used for the positive control Triplicate 7.0 ml portions of SWFI were prepared to be tested as direct contact. Triplicate 10 ml portions of SWFI were prepared and subjected to the extraction conditions previously described for the test article.
Stability Testing:	Marketed product stability characterized by its labeling
Strength, Purity and Composition:	Strength: Not applicable no active components in the formulation; Purity: Meets requirements of USP <643> Total Organic Carbon and USP <645> Water Conductivity Grade and USP <85> Bacterial Endotoxin testing and is certified as USP grade; Composition: Neat CAS #: 7732-18-5.
Blank Preparation:	CMF-PBS was used as the blank. Triplicate 7.0 ml portions of CMF-PBS were prepared to be tested direct contact. Triplicate 10 ml portions of CMF-PBS were subjected to the extraction conditions previously described for the test article
Condition of Extracts:	Test: clear Negative Control: clear Positive Control: clear Blank: clear

3. Test System

Test System
Whole blood samples for use in this test were collected from the rabbits into EDTA vacuum tubes.

Breed:	New Zealand White
Sex:	Male
Estimated Date of Birth:	June 30, 2008

Justification of Test System
Hemolysis testing of medical device materials has historically been used to measure blood compatibility *in vitro*.

4. Preparation of Standards and Controls

Dilution Factors for Calculations
Drabkin's reagent was used as the hemoglobin reagent throughout the study. Throughout the course of the study, several dilutions of the whole blood or the blood plasma were conducted. To account for these in the calculations, the following dilution factors (DF) were used:

Plasma Hemoglobin Determination:

750 µl of plasma added to 750 µl of hemoglobin reagent

$$DF = \frac{\text{Final volume}}{\text{Volume plasma}} = \frac{1500\ \mu l\ \text{solution}}{750\ \mu l\ \text{plasma}} = 2$$

Whole Blood Hemoglobin Determination:

20 µl of whole blood added to 5 mL of hemoglobin reagent

$$DF = \frac{\text{Final volume}}{\text{Volume blood}} = \frac{5.02\text{ mL solution}}{0.02\text{ mL blood}} = 251$$

Diluted Blood Hemoglobin Determination:

400 µl diluted blood added to 5 mL of hemoglobin reagent

$$DF = \frac{\text{Final volume}}{\text{Volume diluted blood}} = \frac{5.4\text{ mL solution}}{0.4\text{ mL diluted blood}} = 13.5$$

Sample Hemoglobin Determination:

1.0 mL of Supernatant added to 1.0 mL of hemoglobin reagent

$$DF = \frac{\text{Final volume}}{\text{Volume supernatant}} = \frac{2.0\text{ mL solution}}{1.0\text{ mL supernatant}} = 2$$

Total Hemoglobin Concentration in each tube:

1.0 mL of diluted blood added to 7.0 mL CMF-PBS

$$DF = \frac{\text{Final volume blood/PBS - CMF}}{\text{Volume diluted blood}} = \frac{8.0\text{ mL}}{1.0\text{ mL}} = 8$$

Standards Preparation
The Human Hemoglobin Standard was dissolved in hemoglobin reagent. The reconstituted standard was tested at the following concentrations: 1.44, 0.800, 0.600, 0.300, 0.150, 0.0750, 0.0375, and 0.0188 mg/mL. The absorbances of the concentrations were read against a hemoglobin reagent blank in a spectrophotometer set at a wavelength of 540 nm. Using the information obtained from the absorbance readings and concentrations, a standard curve was generated.

Plasma Hemoglobin Determination
A 3.0 mL aliquot of the anticoagulated pooled rabbit blood was centrifuged at 700-800 Xg for 15 minutes. A 750 µl portion of the plasma (supernatant) was added to 750 µl of hemoglobin reagent. The solution was allowed to stand for 15 minutes at room temperature and the absorbance was read at 540 nm. The plasma hemoglobin concentration of the blood sample was calculated from the prepared standard curve. If the plasma hemoglobin was greater than or equal to 2.0 mg/mL, the blood was not used for the study.

Blood Hemoglobin Determination
Duplicate 20 µl portions of well-mixed, pooled whole blood (plasma hemoglobin < 2.0 mg/mL) were added to 5.0 mL aliquots of hemoglobin reagent. These solutions were allowed to stand for 15 minutes at room temperature and then the absorbances were read at 540 nm. The whole blood hemoglobin concentration was calculated from the prepared standard curve.

The hemoglobin concentration of the pooled blood sample was adjusted to 10 ± 1.0 mg/mL by diluting with an appropriate amount of CMF-PBS. The hemoglobin concentration was confirmed by taking 400 µl of the well-mixed, diluted blood and adding it to 5.0 mL of hemoglobin reagent in triplicate. The solutions were allowed to stand at room temperature for 15 minutes and the absorbances were read at 540 nm. The diluted blood hemoglobin concentration of the sample was calculated from the prepared standard curve.

5. Method

Clot-free blood samples were collected from each rabbit into 7.0 mL EDTA vacuum tubes containing 12 mg of EDTA on the same day as the test was performed. The blood collected from each rabbit was pooled into a borosilicate screw cap tube and mixed gently to prevent mechanical hemolysis.

The pooled rabbit blood was diluted with CMF-PBS to a total hemoglobin concentration of 10 ± 1.0 mg/mL. Based on a ratio of 1.0 mL diluted blood to 7.0 mL vehicle, the following tubes were prepared:

Direct Contact

18.8 mL of diluted blood and the test article in 131.2 mL CMF-PBS.

1.0 mL of diluted blood and the negative control in 7.0 mL CMF-PBS

1.0 mL of diluted blood and 7.0 mL of SWFI as the positive control

1.0 mL of diluted blood and 7.0 mL CMF-PBS (blank)

Extraction

1.0 mL of diluted blood and 7.0 mL of a test article CMF-PBS extract

1.0 mL of diluted blood and 7.0 mL of a negative control CMF-PBS extract

1.0 mL of diluted blood and 7.0 mL of a positive control SWFI extract

1.0 mL of diluted blood and 7.0 mL of a blank CMF-PBS extract.

The tubes were capped, inverted gently to mix the contents, and then maintained for 3 hours at 37°C with periodic inversions. Following incubation, the blood-CMF-PBS mixtures were transferred to separate disposable centrifuge tubes. These tubes were centrifuged for 15 minutes at 700-800Xg. A 1.0 mL aliquot of each test article, negative control, positive control, and blank supernatant was added to individual 1.0 mL portions of Drabkin's reagent and allowed to stand for 15 minutes at room temperature. The absorbance of each test article, negative control, positive control, and blank solution was measured at 540 nm. The hemoglobin concentration of each test article, negative control, positive control and blank solution was then calculated from the standard curve. The blank corrected percent hemolysis was calculated for each test article and the negative and positive controls as follows:

$$\text{Blank Corrected \% Hemolysis} = \frac{\text{ABS(Sample)} - \text{ABS(Blank)}}{(0.844)\,\text{ABS(Diluted Blood)} - \text{ABS(Blank)}} \times 100$$

ABS = Absorbance

6. Evaluation and Statistical Analysis

The mean blank corrected % hemolysis was calculated by averaging the blank corrected % hemolysis values determined for each of the triplicate test samples. This value is reported to the nearest tenth%. The standard deviation for the replicates was also determined.

An average hemolytic index of the triplicate test samples was also calculated compared to the negative control. A hemolytic index of less than 2% was considered to be non-hemolytic. A hemolytic grade was assigned based on the following scoring scheme:

Hemolytic Index	Hemolytic Grade
0 - < 2%	Non-Hemolytic
2 - 5%	Slightly Hemolytic
> 5%	Hemolytic

For the suitability of the system to be confirmed, the negative control must have had a blank corrected % hemolysis value < 2% and the positive control must have had a blank corrected % hemolysis value of ≥ 8%. If either of these values were not within the acceptable range, the test was repeated with fresh rabbit blood.

7. Results

The values obtained in this study are summarized below:

TEST AND CONTROL DIRECT CONTACT SAMPLES

Sample	ABS 1	ABS 2	ABS 3	Mean Blank Corrected % Hemolysis	Standard Deviation	Mean Concentration (mg/mL)	Hemolytic Index†
Test Article	0.005	0.005	0.005	0	0.0	0.02	0
Negative Control	0.008	0.009	0.008	0.4	0.1	0.03	
Positive Control	0.381	0.400	0.407	98.5	3.4	1.23	
Blanks	0.006	0.007	0.007	1.7*	0.1		

TEST AND CONTROL EXTRACT SAMPLES

Sample	ABS 1	ABS 2	ABS 3	Mean Blank Corrected % Hemolysis	Standard Deviation	Mean Concentration (mg/mL)	Hemolytic Index†
Test Article	0.006	0.005	0.005	0.3	0.1	0.02	0
Negative Control	0.005	0.005	0.005	0.2	0.0	0.2	
Positive Control	0.397	0.433	0.408	102.7	4.6	1.28	
Blanks	0.005	0.004	0.004	1.1*	0.1		

= Not Applicable

*Mean % Hemolysis

†Hemolytic Index calculated as follows:

Test article mean blank corrected % hemolysis – Negative control mean blank corrected % hemolysis

8. Conclusion

Under the conditions of this study, the mean hemolytic index for the test article in CMF-PBS was 0%, and the mean hemolytic index for the CMF-PBS test article extract was 0%. The test article in direct contact was nonhemolytic and the test article extract was nonhemolytic. The negative and positive controls performed as anticipated.

Results and conclusions apply only to the test article tested. Any extrapolation of these data to other samples is the sponsor's responsibility. All procedures were conducted in conformance with good manufacturing practices and 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 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).

American Society for Testing and Materials (ASTM) F756, Standard Practice for Assessment of Hemolytic Properties of Materials (2000).

International Organization for Standardization (ISO) 10993-4, Biological Evaluation of Medical Devices - Part 4: Selection of Tests For Interactions With Blood (2002).

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.

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GLP SAMPLE SUBMISSION FORM

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*Asterisks a required field

08T-48893

Materials List

This listing comprises 'wet' parts only, i.e. parts that come into contact with IV fluid during normal use.

Part Number and Name	*Material*
ALT002-0110 Dual-Outlet Spike	White ABS, Manufacturer: Chi Mei Corporation, Taiwan, Product Code: PA-757
ALT002-0113 Top Cap	
ALT002-0129 Alignment Piece	
ALT002-0118 Dropper Support	
ALT002-0117 Float Guide	
ALT002-0116 Float Body	
ALT002-0128 Float Bottom	
ALT002-0131 Bottom Cap	
ALT002-0120 Upper Dropper Tube	ASTM 304 S30400 Stainless steel tubing
ALT002-0119 Lower Dropper Tube	
ALT002-0166 Spike Cap	PVC – Taizhou Boren Plastic Products Co, Ltd. China – Grade MT-2 – Note: contains DEHP
ALT002-0114 Spike Port	
ALT002-0121 Inlet Tube	
ALT002-0122 Bypass Tube	
ALT002-0130 Central Tube	
ALT002-0115 Extruded Main Chamber	
ALT002-0041 Float Seal	Silicone Rubber - Wacker Elastosil R 401/20
ALT002-0096 Glue	99.5% Cyclohexanone ($C_6H_{10}O$) glue/solvent (cured/dry) - Jiangsu Tengxing chemical
ALT002-0159 Swabbable Needle-free injection Port	OEM – Halkey-Roberts part # 245204024 Polycarbonate: Clear polycarbonate Makrolon RX1805-451118 Silicone: Silicone 40 durometer, blue; Elastosil LR 3003140, OT color K-75238 Blue
ALT002-0105 Air Vent subassembly.	OEM – PVC + hydrophobic filter. Both materials with predicate use.

Geoff Daly, Operations Manager, Analytica Ltd
AUTHORIZED BY SPONSOR — DATE 9 Sept 2008.

NAMSA STUDY DIRECTOR — DATE 9-15-08

REV091107

Statement of Quality Assurance Activities

Phase Inspected	Auditor	Date
Testing	L. M. Byrd	October 9, 2008
Study Data Review	C. M. McCoy	October 14, 2008
Final Report Review	K. J. Evener	October 20, 2008

Reports to Management and Study Director(s)	Date
Periodic Status Report	October 10, 2008

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

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

QA Representative: Karen J. Evener — 10-20-08

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

Date

NAMSA

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08T_48893
26068_001 26068

*Annotates a required field

SPONSOR FINAL REPORT WILL BE ADDRESSED AND MAILED TO

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Brisbane — QLD — 4113
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+61 (7) 3278-1950
PHONE*
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FAX*
GDALY@ANALYTICAMEDICAL.COM
E-MAIL*

INVOICE INFORMATION
As Above
BILLING ADDRESS (include Company Name if different from mailed to)*

7233
PURCHASE ORDER NUMBER*

COST ESTIMATE AND PROPOSAL NUMBER

☐ VISA ☐ MasterCard ☐ American Exp.
CARD HOLDER NAME

CREDIT CARD NUMBER — **EXPIRATION DATE**
+61 (7) 3295-0507 — As Above
ACCOUNTS PAYABLE PHONE* — **ACCOUNTS PAYABLE FAX***

Analytica AutoStart 150mL Burette
TEST ARTICLE NAME *USE EXACT WORDING DESIRED ON FINAL REPORT* * +
As per GMDN code 12159 - Intravenous administration set, general-purpose

INTENDED CLINICAL USE OF TEST ARTICLE:*

☐ BATCH ☐ CODE ☑ LOT — 20080909.
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:* 25 units total (includes non-GLP test units)
(please specify quantities for each lot/batch/code provided)
Single-use, sterile, medical device. Predominantly transparent PVC and white ABS
PHYSICAL DESCRIPTION OF TEST ARTICLE (Chemical/Material type/Color)*

TEST ARTICLE IS CATEGORIZED AS BEING A (check all that apply):* +
X 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:*
X 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*
X ROOM TEMPERATURE ☐ REFRIGERATION ☐ FREEZER
☐ OTHER

① compeeted by JBOll on 9-15-08
JBOll 9-15-08

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 Characterization sections below.

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

Test Article	Control Article	Characterization (if not applicable state clearly the reason why)
① ☒	☐	Strength: N/A: No active ingredients are used to formulate a concentration
① ☒	☐	Purity: N/A because test article is a multi-component device with no active ingredients JBOll 9-15-08
X	☐	Composition: Refer attached materials list.

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

T091508_017
UPS linhai univer start

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*Annotates a required field

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Materials List

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This listing comprises 'wet' parts only, i.e. parts that come into contact with IV fluid during normal use.

Part Number and Name	*Material*
ALT002-0110 Dual-Outlet Spike	White ABS, Manufacturer: Chi Mei Corporation, Taiwan, Product Code: PA-757
ALT002-0113 Top Cap	
ALT002-0129 Alignment Piece	
ALT002-0118 Dropper Support	
ALT002-0117 Float Guide	
ALT002-0116 Float Body	
ALT002-0128 Float Bottom	
ALT002-0131 Bottom Cap	
ALT002-0120 Upper Dropper Tube	ASTM 304 S30400 Stainless steel tubing
ALT002-0119 Lower Dropper Tube	
ALT002-0166 Spike Cap	PVC – Taizhou Boren Plastic Products Co, Ltd. China – Grade MT-2 – Note: contains DEHP
ALT002-0114 Spike Port	
ALT002-0121 Inlet Tube	
ALT002-0122 Bypass Tube	
ALT002-0130 Central Tube	
ALT002-0115 Extruded Main Chamber	
ALT002-0041 Float Seal	Silicone Rubber - Wacker Elastosil R 401/20
ALT002-0096 Glue	99.5% Cyclohexanone ($C_6H_{10}O$) glue/solvent (cured/dry) - Jiangsu Tengxing chemical
ALT002-0159 Swabbable Needle-free injection Port	OEM – Halkey-Roberts part # 245204024 Polycarbonate: Clear polycarbonate Makrolon RX1805-451118 Silicone: Silicone 40 durometer, blue; Elastosil LR 3003140, OT color K-75238 Blue
ALT002-0105 Air Vent subassembly.	OEM – PVC + hydrophobic filter. Both materials with predicate use.

Geoff Daly, Operations Manager, Analytica Ltd
AUTHORIZED BY SPONSOR — DATE 9 SEPT 2008.

NAMSA STUDY DIRECTOR — DATE 9-16-08

GLP PROTOCOL

TEST FACILITY:

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Northwood, OH 43619

SPONSOR:

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Australia

STUDY TITLE:

ASTM Hemolysis

Lab No.: 08T-48893 0

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Approvals

Sponsor Representative (Sponsor):	 Geoff Daly, Operations Manager Analytica Ltd.
Date Approved:	Thursday, 4th September 2008
Study Director (NAMSA):	
Date Initiated:	9-15-08

1. Introduction

Purpose
This procedure describes the method used to determine whether an extract of a biomaterial or a medical device causes hemolysis (lysis of red blood cells) per ASTM F756, Standard Practice for Assessment of Hemolytic Properties of Materials. This study will be based on the requirements of the International Organization for Standardization: Biological evaluation of medical devices, Part 4: Selection of Tests for Interactions with Blood.

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:

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

___ Material thickness less than 0.5 mm - ratio of 60 cm^2:10 mL (based on the USP ratio 120 cm^2:20 mL)
___ Material thickness greater than or equal to 0.5 mm - ratio of 30 cm^2:10 mL (based on the USP ratio 60 cm^2:20 mL)
___ Irregularly shaped objects and/or sponsor option - ratio of 2 g:10 mL (based on the USP ratio 4 g:20 mL)
X Other (explain): Fill Device Wet internal surface area = approx 41127 mm^2, Fluid volume = 170mL total (tubes and chambers)

dB 9-15-08

NOTE: The test article will be prepared in triplicate for both extraction and direct contact; therefore, the amount of test article required is six times that indicated above.

completed by sponsor dB 9-15-08

Test Article Preparation Instructions:

Refer to attached product labelling (file: ALT002-0082-200807232018.pdf). Open the air vent (item "E"). Open clamps C and D. Fill main chamber to approximately half way and close off clamps. With thumb and forefinger squeeze the pvc spike port (the small chamber to the right of label "G" in the diagram) until approximately almost full. Fluid will enter the chamber via the canula. Reopen the clamps and allow the fluid to flood the chamber. Close the vent(E). The item can now be exposed to the extraction conditions. To remove the extraction vehicle, open the air vent, and either puncture the diaphragm at the spike port (H), AND/OR the clamps opened and the device turned upside down, AND/OR the device may be punctured or otherwise destroyed or opened.

Vehicle: Calcium and magnesium free phosphate buffered saline (CMF-PBS)

The extraction conditions should not in any instance cause physical changes such as fusion or melting, with results in a change in the available surface area. A slight adherence of the pieces can be tolerated.

Extraction Conditions (select one):

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

Disposition of Test/Control Article (select one):

X Discard ___ Return unused article ___ Return unused and used article

completed by Sponsor dB 9-15-08

Special Laboratory Instructions:

No special instructions from Sponsor

Control Article

Negative Control: High density polyethylene will be prepared in triplicate using a ratio of 60 cm^2:20 mL for direct contact and extraction in CMF-PBS using the same extraction conditions as described for the test article.

Positive Control: A 10 mL aliquot of Sterile Water for Injection (SWFI) prepared in triplicate using the extraction conditions as described for the test article will be used as the extraction control. Triplicate 7.0 mL aliquots of SWFI will be used as the direct contact control.

3. Test System

Test System

Whole blood samples for use in this test will be collected from the rabbits in EDTA vacuum tubes. A sample of clot-free blood will be collected from each rabbit and the tubes will be pooled in a borosilicate screw cap tube and mixed gently to prevent hemolysis.

Breed: New Zealand White
Sex: No particular gender is prescribed for this test
Age: No particular age is prescribed for this test

Justification of Test System

Hemolysis testing of medical device materials has historically been used to measure blood compatibility *in vitro*.

4. Preparation of Standards and Controls

Dilution Factors For Calculations

Throughout the course of this study, several dilutions of the whole blood or the blood plasma will be conducted. To account for these dilutions in the calculations that will be performed, the following dilution factors (DF) will be used:

Plasma Hemoglobin Determination:

750 µl of plasma added to 750 µl of Drabkin's or Cyanmethemoglobin reagent

$$DF = \frac{\text{Final volume}}{\text{Volume plasma}} = \frac{1500\,\mu\text{l solution}}{750\,\mu\text{l plasma}} = 2$$

Whole Blood Hemoglobin Determination:

20 µl of whole blood added to 5 mL of Drabkin's or Cyanmethemoglobin reagent

$$DF = \frac{\text{Final volume}}{\text{Volume blood}} = \frac{5.02\,\text{mL solution}}{0.02\,\text{mL blood}} = 251$$

Diluted Blood Hemoglobin Determination:

400 µl diluted blood added to 5 mL of Drabkin's or Cyanmethemoglobin reagent

$$DF = \frac{\text{Final volume}}{\text{Volume diluted blood}} = \frac{5.4\,\text{mL solution}}{0.4\,\text{mL diluted blood}} = 13.5$$

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Sample Hemoglobin Determination:

1.0 mL of Supernatant added to 1.0 mL of Drabkin's or Cyanmethemoglobin reagent

$$DF = \frac{\text{Final volume}}{\text{Volume supernatant}} = \frac{2.0\text{ mL solution}}{1.0\text{ mL supernatant}} = 2$$

To Determine Total Hemoglobin present in each tube:

1.0 mL of diluted blood added to 7.0 mL CMF-PBS

$$DF = \frac{\text{Final volume blood/PBS - CMF}}{\text{Volume diluted blood}} = \frac{8.0\text{ mL}}{1.0\text{ mL}} = 8$$

Standards Preparation

The Human Hemoglobin Standard will be reconstituted in Drabkin's or Cyanmethemoglobin reagent at a concentration of 1.44 mg/mL. The reconstituted standard may be stored at 5 ±3°C for up to 2 months from the date of preparation. The reconstituted standard will be diluted to 0.800, 0.600, 0.300, 0.150, 0.0750, 0.0375, and 0.0188 mg/mL. The absorbances of the dilutions and the starting standard solution will be read against a Drabkin's or Cyanmethemoglobin reagent blank at a wavelength of 540 nm. Using the information obtained from the absorbance readings and concentrations, a standard curve will be generated by linear regression.

Plasma Hemoglobin Determination

A 3 mL aliquot of the anticoagulated pooled rabbit blood will be centrifuged at 700-800 Xg for 15 minutes. A 750 µl portion of the plasma (supernatant) will be added to 750 µl Drabkin's or Cyanmethemoglobin reagent. The solution will be allowed to stand at room temperature for 15 minutes for Drabkin's reagent or 5 minutes for Cyanmethemoglobin reagent and then the absorbance (A) of the solution will be read at a wavelength of 540 nm. The plasma hemoglobin concentration in the blood sample will be calculated as follows using the slope of the standard curve.

Plasma Free Hemoglobin Concentration (PFH) = A (Plasma) * Slope * 2 (DF)

If the plasma hemoglobin is greater than 2 mg/mL, the blood will not be used for this test.

Blood Hemoglobin Determination

A 20 µl portion of well-mixed, pooled whole blood (plasma hemoglobin ≤2 mg/mL) will be added to 5 mL of Drabkin's or Cyanmethemoglobin Reagent. This solution will be allowed to stand at room temperature for 15 minutes for Drabkin's Reagent or 5 minutes for Cyanmethemoglobin reagent and then the absorbance of the solution will be read at 540 nm. The hemoglobin concentration of the pooled blood sample will be calculated as follows using the slope of the standard curve.

Whole Blood Hemoglobin Concentration (WB) = A * Slope * 251(DF)

The total hemoglobin content of the pooled blood sample will be adjusted to 10 ± 1 mg/mL by diluting with an appropriate amount of CMF-PBS. The hemoglobin concentration will be confirmed by taking 400 µl of the well-mixed, diluted blood and adding it to 5 mL of Drabkin's or Cyanmethemoglobin Reagent. This solution will be allowed to stand at room temperature for 15 minutes for Drabkin's reagent or 5 minutes for Cyanmethemoglobin reagent and then the absorbance of the solution will be read at 540 nm. The concentration of hemoglobin in the diluted blood will be calculated as follows using the slope of the standard curve.

Diluted Blood Hemoglobin Concentration (DB) = A * Slope * 13.5(DF)

This number will be divided by 8 (DF) to obtain the hemoglobin concentration present in each tube.

5. Method

Following the extraction procedure, the appropriate volume of diluted blood (based on a ratio of 1 mL diluted blood/7 mL vehicle) will be added to the following: triplicate tubes of the test, negative control, positive control, and blank extracts as well as triplicate tubes of the test, negative control, positive control, and blanks in direct contact. The tubes will be maintained for at least 3 hours at 37 ± 2°C with gentle periodic inversions. Following incubation, all tubes will be centrifuged at 700-800 Xg for 15 minutes.

NOTE: At this point, samples may be decanted, capped and stored in a freezer for up to 96 hours before subsequent hemoglobin analysis.

A 1 mL portion of each supernatant will be added to separate tubes containing 1 mL of Drabkin's or Cyanmethemoglobin reagent. The test and control solutions will stand at room temperature for 15 minutes for Drabkin's reagent or 5 minutes for Cyanmethemoglobin reagent and the absorbances will be read at 540 nm.

6. Evaluation and Statistical Analysis

Using the slope of the standard curve, the hemoglobin concentration in each sample supernatant (hemoglobin released) will be determined as follows:

Supernatant Hemoglobin Concentrations = A * Slope * 2 (DF). The blank corrected percent hemolysis will be calculated for each test article solution and the negative and positive controls as follows:

$$\text{Blank Corrected \% Hemolysis} = \frac{\text{ABS(Sample)} - \text{ABS(Blank)}}{(0.844)\text{ABS(Diluted Blood)} - \text{ABS(Blank)}} \times 100$$

The mean blank corrected % hemolysis will be calculated by averaging the blank corrected % hemolysis values determined for each of the triplicate test samples. This value will be reported to the nearest 1%. The standard deviation for the replicates will also be determined.

An average hemolytic index of the triplicate test samples will also be calculated compared to the negative control. A hemolytic index of 2% or less will be considered to be nonhemolytic. A hemolytic grade will be assigned based on the following scoring scheme:

Hemolytic Index	Hemolytic Grade
0 - 2%	Nonhemolytic
2 - 5%	Slightly Hemolytic
> 5%	Hemolytic

For the suitability of the system to be confirmed, the negative control must have a blank corrected % hemolysis value < 2% and the positive control must have a blank corrected % hemolysis value of ≥ 8%. If either of these values is not within the acceptable range, the test will be repeated with fresh rabbit blood.

7. Report

The final report will include a description of the methods employed, absorbance values/results for the test and controls in direct contact and as extracts, the hemolytic index and grade of the test in direct contact and as an extract, and any additional pertinent observations.

8. Quality Assurance

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

9. Proposed Dates

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

10. Records

Test article and control preparation, the source of the blood used in the test, dates of relevant activities (such as the test initiation and completion), absorbance values, and individual hemoglobin concentration values will be recorded.

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

11. References

21 CFR 58 (GLP Regulations).

American Society for Testing and Materials (ASTM) F756, Standard Practice for Assessment of Hemolytic Properties of Materials (2000).

International Organization for Standardization (ISO) 10993-4, Biological Evaluation of Medical Devices - Part 4: Selection of Tests For Interactions With Blood (2002).

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.

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September 17, 2008

Geoff Daly
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Australia

PROTOCOL AMENDMENT I

Test Article: Analytica AutoStart 150mL Burette

Identification: Lot: 20080909

NAMSA Submission ID.: 08T_48893

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:
V0014_130	08T_48893_02	Cytotoxicity Study Using the ISO Elution Method - 1X MEM Extract	October 1, 2008	October 9, 2008
TI261_300	08T_48893_03	ISO Maximization Sensitization Study - Extract - 0.9% SC Extract	September 27, 2008	November 19, 2008
TI251_800	08T_48893_04	ISO Intracutaneous Study - Extract - 0.9% SC Extract	September 21, 2008	October 15, 2008
T0625_500	08T_48893_05	ISO Systemic Toxicity Study - Extract - 0.9% SC Extract	September 22, 2008	October 15, 2008
V0607_100	08T_48893_06	ASTM Hemolysis - CMF-PBS Extract	October 16, 2008	October 20, 2008

Jolee Bartrom, B.S.
Study Director

9-17-08
Date

cc: QA (NAMSA)
Sponsor

NAMSA

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October 8, 2008

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REVISED*
PROTOCOL AMENDMENT I

Test Article: Analytica AutoStart 150mL Burette

Identification: Lot: 20080909

NAMSA Submission ID.: 08T_48893

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T0625_500	08T_48893_05	ISO Systemic Toxicity Study - Extract - 0.9% SC Extract	September 22, 2008	October 15, 2008
V0607_100	08T_48893_06	ASTM Hemolysis - CMF-PBS Extract	October 16, 2008	October 20, 2008

*This amendment has been revised to correct the sponsor's address.

Jolee Bartrom

Jolee Bartrom, B.S.
Study Director

10-8-08

Date

cc: QA (NAMSA)
Sponsor