GLP REPORT

TEST FACILITY

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SPONSOR

Geoff Daly
Analytica LTD
85 Brandl Street, Eight Mile Plains
Brisbane, Queensland, 4113
Australia

CONFIDENTIAL

STUDY TITLE

Cytotoxicity Study Using the ISO Elution Method - 1X MEM Extract

TEST ARTICLE NAME

Analytica AutoStart 150mL Burette

TEST ARTICLE IDENTIFICATION

Lot: 20080909

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Summary

This *in vitro* study was conducted to evaluate Analytica AutoStart 150mL Burette, Lot: 20080909, for potential cytotoxic effects following the guidelines of International Organization for Standardization 10993: Biological Evaluation of Medical Devices, Part 5: Tests for Cytotoxicity: *in vitro* Methods. A single preparation of the test article was extracted in Minimum Essential Medium supplemented with 5% serum and 2% antibiotics at 37°C for 24 hours. The negative control, reagent control, and positive control were similarly prepared. Triplicate monolayers of mouse fibroblast cells (L-929) were dosed with each extract and incubated at 37°C in presence of 5% CO_2 for 48 hours. Following incubation, the monolayers were examined microscopically for abnormal cell morphology and cellular degeneration.

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

Study and Supervisory Personnel:

Molly F. Corvo, B.S.
Jordan J. Holton
Christopher T. Lubelski, B.S.
Sean W. Dugan, B.A.
Don R. Pohl, B.S.
Melissa A. Cadaret, B.A., M.S.

Approved by:

Jolee Bartrom

Jolee Bartrom, B.S.
Study Director

10-8-08
Date Completed

Authorization for duplication of this report, except in whole, is reserved pending NAMSA's written approval.

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 10-3-08

Jolee Bartrom, B.S. Date

1. Introduction

Purpose
The test article identified below was extracted and the extract was subjected to an *in vitro* cytotoxicity study to determine whether leachables extracted from the material would cause cytotoxicity.

Testing Guidelines
The testing procedures are based on the requirements of the International Organization for Standardization 10993: Biological Evaluation of Medical Devices, Part 5: Tests for Cytotoxicity: *in vitro* Methods.

Dates

Test Article Receipt:	September 15, 2008
Cells Dosed Date:	September 23, 2008
Observations Concluded Date:	September 25, 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 2.
Physical Description of the Test Article:	Single-use, sterile, medical device. Predominantly transparent PVC and white ABS.
Storage Conditions:	Room Temperature
Extraction Vehicle:	Single strength Minimum Essential Medium supplemented with 5% fetal bovine serum and 2% antibiotics (10 units/mL penicillin, 10 µg/mL streptomycin and 2.5 UG/mL amphotericin B) designated as 1X MEM.
Negative Control Article:	High density polyethylene (HDPE)
Negative Control Article Stability Testing:	Marketed product stability characterized by its labeling.
Negative Control Article Strength, Purity and Composition:	HDPE: 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.

Reagent Control Article: Single strength Minimum essential Medium (1X MEM)

Reagent Control Article Stability Testing: Marketed product stability characterized by its labeling.

Reagent Control Article Strength, Purity and Composition: 1X MEM: Strength: Not applicable, no active components in the formulation; Purity: Not applicable, multi-component article; Composition: 92% Gibco MEM w/Earle's Salts, 5% Fetal Bovine Serum, 2% Antibiotics (Amphotericin, Pen-Strep), and 1% (200 mM) L-Glutamine.

Positive Control Article: Tin stabilized polyvinylchloride (TSV)

Positive Control Article Stability Testing: Marketed product stability characterized by its labeling.

Positive Control Article Strength, Purity and Composition: TSV: Plasticizer, tin stabilized polyvinylchloride and 10, 10'-oxybisphenoxarsine (OBPA).

Extraction Procedure: The air vent (item E), clamp C, and clamp D were opened. The main chamber was filled approximately half way and the clamps were closed. With the thumb and forefinger, the pvc spike port (the small chamber to the right of label "G" in the diagram) was squeezed until approximately almost full. The fluid entered the chamber via the canula. The clamps were reopened and the fluid was allowed to flood the chamber. The vent (E) was closed. The item was now exposed to the extraction conditions. The extraction vehicle was removed by opening the vent and puncturing the diaphragm at the spike port (H) and/or opening the clamps and turning the device upside down, and/or the device was punctured or otherwise destroyed or opened. One device was filled to capacity with a total of 158 ml of the vehicle. The device was sealed as necessary to avoid loss of the vehicle during extraction. A single preparation of the test article and each of the controls were subjected to the following extraction conditions as described below.

Treatment Group	Extraction Ratio	Test/Control Sample Amount	Volume of Extraction Vehicle	Extract Condition
Test Article	NA	NA	NA	37°C for 24 hours
Negative Control	60 cm^2 : 20 mL	30.8 cm^2	10 mL	37°C for 24 hours
Reagent Control	NA	NA	10 mL	37°C for 24 hours
Positive Control	60 cm^2 : 20 mL	60.8 cm^2	20 mL	37°C for 24 hours

The extracts were agitated during extraction.

Extraction Vehicle	Treatment Group	Condition of Extract
1X MEM	Test Article	Clear
1X MEM	Negative Control	Clear
1X MEM	Reagent Control	Clear
1X MEM	Positive Control	Clear

3. Test System

Test System Management

L-929, mouse fibroblast cells, (ECACC Catalog No. 85103115, or equivalent source) were propagated and maintained in single strength Minimum Essential Medium supplemented with 5% fetal bovine serum and 2% antibiotics (10 units/mL penicillin, 10 µg/mL streptomycin and 2.5 UG/mL amphotericin B) at 37°C in a gaseous environment of 5% carbon dioxide (CO_2). For this study, cells were seeded in 10 cm^2 wells and incubated at 37°C with 5% CO_2 to obtain sub-confluent monolayers of cells prior to use. Aseptic procedures were used in the handling of the cell cultures following approved NAMSA Standard Operating Procedures.

4. Method

Triplicate culture wells were selected which contained a sub-confluent cell monolayer. The growth medium contained in the triplicate cultures was replaced with 2 mL of the test extract in each well. Similarly, the growth medium in triplicate 10 cm^2 wells was replaced with 2 mL of the reagent control, negative control and the positive control. The wells were incubated at 37°C in 5% CO_2 for 48 hours.

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

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

The color of the test medium was observed to determine any change in pH. A color shift toward yellow indicates an acidic pH range and a color shift toward magenta to purple indicates an alkaline pH range.

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

5. Results

No cytotoxicity or cell lysis was noted in any of the test wells. No pH shift observed at 48 hours. The reagent control, negative control and the positive control performed as anticipated. The individual reactivity grades are shown in Appendix 1.

6. Conclusion

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

Results and conclusions apply only to the test article tested. Any extrapolation of these data to other samples is the sponsor's responsibility. All procedures were conducted in conformance with good manufacturing practices and certified to ISO 13485:2003.

7. Quality Assurance

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

8. Proposed Dates

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

9. Records

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

10. References

21 CFR 58 (GLP Regulations).

International Organization for Standardization (ISO) 10993-5, Biological Evaluation of Medical Devices - Part 5: Tests for Cytotoxicity, *In Vitro* Methods (1999).

United States Pharmacopeia 31, National Formulary 26 (USP), General Chapter <87>, Biological Reactivity Tests, In Vitro (2008).

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

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

11. Protocol Changes

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

Appendix 1 - Reactivity Grades For Elution Testing

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

Note: A, B and C denote replicates.

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GLP SAMPLE SUBMISSION FORM

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Ohio

*Annotates a required field

08T-48695

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
ALT002-0114 Spike Port	– Note: contains DEHP
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

9 SEPT 2008.
DATE

NAMSA STUDY DIRECTOR

9-15-08
DATE

REV091107

Statement of Quality Assurance Activities

Phase Inspected	Auditor	Date
Dosing	K. J. Evener	September 23, 2008
Study Data Review	S. M. Pelliltieri	September 26, 2008
Final Report Review	K. J. Evener	October 8, 2008

Reports to Management and Study Director(s)	Date
The findings of inspections for this study did not warrant interim reports to management and study director.	Not Applicable

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 — 10-8-08

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

Date

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08T_48893
26068_001 26068

*Annotates a required field

SPONSOR FINAL REPORT WILL BE ADDRESSED AND MAILED TO

ANALYTICA LTD		Geoff Daly
COMPANY NAME*		ATTN*
85 Brandl St, Eight Mile Plains		
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Brisbane	QLD	4113
CITY*	STATE*	ZIP*
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COUNTRY*		
+61 (7) 3278-1950		
PHONE*		
+61 (7) 3259-8313		
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 competed by JB1011 on 9-15-08
JB1011 9-1508

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 X	☐	Strength: N/A: No active ingredients are used to formulate a concentration
① X	☐	Purity: N/A because test article is a multi-component device with no active ingredients JB1011 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

NAMSA

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*Annotates a required field

08T-48095

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

GLP PROTOCOL

TEST FACILITY:

NAMSA
6750 Wales Road
Northwood, OH 43619

SPONSOR:

Geoff Daly
Analytica Ltd
Eight Mile Plains
Brisbane, Queensland 4113
Australia

STUDY TITLE:

Cytotoxicity Study Using the ISO Elution Method

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08T-48893 02

Approvals

Sponsor Representative (Sponsor):	Geoff Daly, Operations Manager Analytica Ltd.
Date Approved:	Friday 5th September 2008
Study Director (NAMSA):	
Date Initiated:	9-15-08

1. Introduction

Purpose
The purpose of this study is to evaluate the biocompatibility of a test article extract using an *in vitro* mammalian cell culture test. This study is based on the requirements of the International Organization for Standardization 10993: Biological Evaluation of Medical Devices, Part 5: Tests for Cytotoxicity: *in vitro* Methods.

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)

Completed by parsa 081011 9-15-08

NOTE: Only a single test article will be prepared.

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.

Extraction Vehicle (select all that apply):

X Single strength Minimum Essential Medium supplemented with 5% serum and 2% antibiotics (1X MEM)
___ Single strength Minimum Essential Medium, serum free, but supplemented with 2% antibiotics (MEM-SF)
___ 0.9% Sodium Chloride Solution , USP (SC)
___ Purified Water (PW)

If an SC or PW extract is prepared, a 50% concentration [1 part test extract to 1 part double strength Minimum Essential Medium supplemented with 10% serum and 4% antibiotics (2X MEM)] of the test extract and 2X MEM will be made prior to dosing the cell monolayers. The negative control and reagent control will be handled in the same manner.

The extraction conditions shall attempt to exaggerate the clinical use conditions so as to define the potential toxicological hazard; however, they should not in any instance cause physical changes such as fusion or melting, which results in a decrease in the available surface area. A slight adherence of the pieces can be tolerated.

Completed by sponsor 08/08/19-15:08

Extraction Conditions (select one):

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

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

Disposition of Test/Control Article (select one):

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

Special Laboratory Instructions:

No special instructions from Sponsor

Control Article

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

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

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

3. Test System

Test System and Justification

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

Test System Management

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

4. Method

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

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

5. Evaluation and Statistical Analysis

The color of the test medium will be observed. Each culture well will be evaluated for percent lysis and cellular characteristics using the following table (direct excerpt from USP):

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

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

6. Report

The final report will include information on the cell line, culture medium methods, test and control results, and any additional pertinent observations.

7. 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.

8. 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).

9. Records

Material preparation, cell line and passage number, control and test well observations, and dates of relevant activities (such as the study initiation and termination) 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.

10. References

Code of Federal Regulations (CFR), Title 21, Part 58, Good Laboratory Practice for Nonclinical Laboratory Studies (2007).

International Organization for Standardization (ISO) 10993-5, Biological Evaluation of Medical Devices - Part 5: Tests For Cytotoxicity, *In Vitro* Methods (1999).

United States Pharmacopeia 30, National Formulary 25 (USP), General Chapter <87>, Biological Reactivity Tests, In Vitro (2007).

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

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

11. Protocol Changes

Any necessary changes to the protocol after sponsor approval or study initiation 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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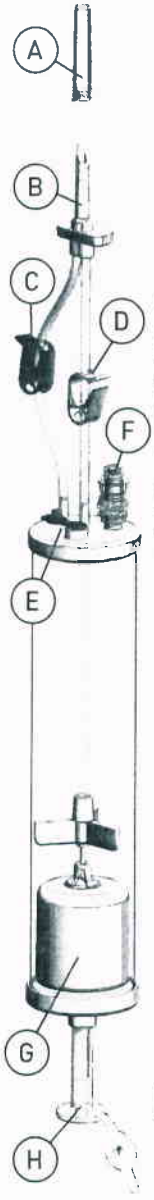
OPEN

ANALYTICA

AutoStart®

STERILE SINGLE-USE 150 mL BURETTE



Setting Up

1. Close the WHITE On/Off clamp (D), and BLUE bypass clamp (C).

2. Open filtered vent (E). NOTE: This vent should be left open during normal use.

3. Remove the spike cap (A)

4. Puncture solution container with spike (B).

5. Open WHITE on/off clamp (D). Fluid will begin to fill the chamber and will be stopped by the float (G).

6. Open the spike port cap (H).

7. Connect an infusion line to the spike port (H).

8. Prime the system according to the infusion line instructions.

• The device is now ready for use.

Medication Delivery

1. Open and shut the BLUE bypass Clamp (C) to fill the Burette with infusion fluid.

2. Add medication via injection site (F) as per hospital protcol.

• The Autostart float (G) will automatically return the device to continuous infusion mode once the medication has been delivered.

⚠ Important Notes

• The float (G) shuts off flow once the fluid reservoir is empty. This shutoff is not for long-term use.

• Replace device every 24 hours or per hospital protocol.

• Sterile whilst packaging intact. Do not use if packaging is damaged or if protective caps are not in place.

• Gravity feed only.

• Use aseptic technique.

• WARNING: Air in infusion line may cause embolism

STERILE EO

NON-PYROGENIC
NON-TOXIC
LATEX FREE

CE 0123

Manufacturer: Zhejiang Lingyang Medical Apparatus Co. Ltd. Baishuiyang, Linhai City Province CHINA www.ly-medical.com LY

TGA Sponsor: Analytica Ltd. 85 Brandl St. Eight Mile Plains, Brisbane, 4113 AUSTRALIA www.**AutoStartBurette**.com

ALT002-0082-v1

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LOT

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September 17, 2008

Geoff Daly
Analytica Ltd
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Brisbane, Queensland, 4113
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

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*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