Transducer Protector

Hemolysis Test

FINAL REPORT

Client: Finetech Research and Innovation Corporation
Testing Institution: SGS Taiwan Ltd
Report No. : UB/2013/70737A-02
Report Date: 2013/09/06

Note:

1. The content of this report is invalid if it is not presented as the entire report.
2. Any unauthorized alteration, forgery or falsification of the content or appearance of this report is unlawful and offenders may be prosecuted to the fullest extent of the law.
3. The results shown in this test report refer only to the test article(s) tested.
4. The report is the Chinese version of translations UB/2013/70737A-03

STUDY SCHEDULE
Hemolysis Test:
Transducer Protector

Report No.:	UB/2013/70737A-02
Study Initiation date:	2013/08/27
Experimental starting date:	2013/08/27
Experimental completion date:	2013/09/04
Study completion date:	See Study Director's signature date in the report
Name of study personnel:	Jeff Chen

Testing Institution

Name: SGS TAIWAN LTD.

Address: No. 38, Wu Chyuan 7th Rd., New Taipei Industrial Park, Wu Ku Dist., New Taipei City 24890, Taiwan (R. O. C.)

Client / Sponsor

Name: Finetech Research and Innovation Corporation

Address: No.29, Anle St., Xiushui Township, Changhua County 504, Taiwan (R.O.C.)

TEST ARTICLE INFORMATION

INFORMATION FOR ☒ TEST ARTICLE/ ☐ CONTROL ARTICLE

Sponsor Company Name	Finetech research and innovation corporation
Sponsor Address	No.29, Anle St., Xiushui Township, Changhua County 504, Taiwan (R.O.C.)
Contract study item	☒ Base on the contract ☐ Others _____
Name of Test article/ Control article	Transducer Protector
Batch/Lot number	☐ Base on the specific number on the package : _____ ☐ Base on the date on the package : _____ ☐ Base on the arrived date ☑ Others : N/A
Specification & Amount	10pcs/pack * 7packs (e.g.10ml / bottle * 6 bottles)
Retention amount (Note 2)	The amount of the same lot is sufficient for ☐ One test ☐ Two test (for retention)
External features	External features: ☐ liquid ☐ powder ☐ tablet ☐ capsule ☒ Other column — Color : translucent white
Major components & Purity	Major components: Polypropylene meterial housing with membrane — Purity: _____
Solvent and solubility	N/A
Storage condition	☒ Room temperature ☐ 4℃ ☐ Dry ☐ Light sensitive ☐ Others _____
Expiration date(Note 3)	☐ Date: ___ / ___ / ___ (YYYY/MM/DD) or ☒ Period : 2 year 0 month 0 day
Attachment(Note 4)	☐ Certificate of Analysis ☐ Material Safety Data Sheet ☐ Stability Test Result ☐ Other : _____ ☒ No attachment (Note4)
Sterilization	Has been sterilized ☐ YES ☒ NO (If Yes, please select the following item) Methods ☐ EOsterilization ☐ Gamma sterilization ☐ Steam sterilization ☐ Other _____
Categorization of devices (The column is only for device used)	1. ☒ Contact with intact skin or mucosa (cumulative contact duration) ☒ Short-term (no greater than 4 hr) ☐ Long-term (exceeding 4 hr) Maximum duration is _____ hrs 2. ☐ Implanted device
Specific requirement (Note 5)	N/A

Sponsor Signature/ Date : Golden Li 2013. July 12th.

Note 1. Above all information is disclosure by the sponsor.
Note 2. If the sponsor doesn't provide the retention of test article/control article, the retention of a reserved test article/control article from each batch of test article /control article is the responsibility of the Sponsor.
Note 3. If the effective period is less than 5 years, the test article/control article will be retained till the expiry date. If the effective period is longer than 5 years, the test article/control article will be retained for 5 years only.
Note 4. Determination and documentation of identity, strength, purity, stability, composition, method of synthesis, fabrication, derivation or other characteristics of the test article/control article are the responsibility of the Sponsor.
Note 5. The test article/control article which has been destroyed or cutting will be discarded after the end of experiment. For retention or return of the kind of test article/control article, please indicate in the "special requirement". The human intake suggests or dose requested by the sponsor also can fill in the "special requirement". Note treatment method after test if the test article need to be retreated
Note 6. The code number of test article is the same as the report number.
Note 7. Note 'N/A' if not applicable. Do not leave blank.

版次：3.1 試驗-對照物質資料表 Information for test article-control article
發行日期：2013.06.14

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SGS

STATEMENT OF GLP COMPLIANCE

All study activities performed by SGS Taiwan are carried out in compliance with the GLP (Good Laboratory Practices) for Nonclinical Laboratory Studies (Department of Health, Taiwan, 2006), current OECD Principles of Good Laboratory Practice (Organization for Economic Cooperation and Development, Paris, ENV/MC/CHEM (98) 17) and U.S. Food and Drug Administration Good Laboratory Practice Regulations, 21 CFR Part 58. (1987).The study was conducted in accordance with the protocol and standard operating procedures and monitored in conformity with the protocol. All laboratory data are accurately recorded and verified. SGS Taiwan makes no GLP compliance claim for characterization and verification of the test article identity and properties; this is the responsibility of the sponsor.

Study Director:

Howard Kao — 2013. 10. 23

Howard Kao / SGS Taiwan Ltd. — Date Completed

Deputy of
Facility Manager: Amy Liu — 2013. 10. 23

Amy Liu / SGS Taiwan Ltd. — Date Completed

SGS

QUALITY ASSURANCE STATEMENT

UB/2013/70737A-02
Transducer Protector
Hemolysis Test

This study was audited by Quality Assurance personnel of SGS Life Science Service. The QA inspection report includes review of study plan, result of a study-based audit and results of audit of raw data and study report. The audit report was issued upon the completion of final report of testing.

QA:

Melissa Lin / SGS Taiwan Ltd.

2013. 10. 22

Date Completed

Inspection Type	Inspection date	Study phase	Date to facility manager and study director
Study base	2013/08/27	Draft Protocol	2013/08/27
Study base	2013/08/30	Absorbance of solution detection	2013/08/30
Study base	2013/09/16	Raw data & Draft Final report	2013/09/16

ARCHIVING

All the study-related raw data, records, protocol and the final report will be kept in archives room of SGS (TAIWAN) LTD for 5 years. Furthermore, retention of the test articles will be in Sample Storage Room until its expiration date or up to 5 years. All of the records and test articles are handled according to GLP guideline. Agent authorized by the sponsor can apply for the review according to SGS procedure.

Address:

No. 38, Wu Chyuan 7th Rd., New Taipei Industrial Park, Wu Ku Dist., New Taipei City 24890, Taiwan (R. O. C.)

Archiving List	
Final report	Final Report Copy
Raw Data	Hemolysis Test Data Sheet
Records	Application Form Information for test article-control article GLP Test Article Control From and other supplementary record
Protocol	Protocol

ABSTRACT

Hemolysis test was the objective study to determine the hemolytic properties under static condition with extract of the test articles and the test article direct when contacted with blood. The study was performed according to the guideline ISO 10993-4 and ASTM F756. The percent hemolysis of the test article "Transducer Protector" extract was 1.07 % in extract test; the percent hemolysis of the test article was 0.134 % in direct contact test. Therefore, base on the hemolytic index, the "Transducer Protector" was non-hemolytic.

PURPOSE

For decades, the *in vitro* hemolysis test has been used to identify the biocompatibility properties of medical device. Interaction of red blood cells (RBC) with medical device or with a medical device extract can cause the release of intracellular hemoglobin. Multiple factors can induce hemolysis such as shear stress, interaction of RBC with leachables, chemicals, electrical forces and metal ions. Hemolytic test is the objective study to determine the hemolytic property under static condition with extract of the "Transducer Protector" when contact with blood. Therefore, the test system is blood for the test. The hemolysis test *in vitro* mimics the *in vivo* situation concerning blood source, incubation temperature, blood dynamics and the absence of air. The study is performed based on the guideline ISO 10993-4 and ASTM F 756.

EXPERIMENTAL DESIGN

1. Test system:

A. Blood : From Rabbit

B. Blood Source: LEON Biotech. Co. Ltd. Biocompatibility Laboratory

2. Reagents

A. Potassium phosphate (Sigma, Cat No. 30407, Lot No. SZBA1890)

B. Potassium cyanide (Alfa Aesar, Cat No. L13273, Lot No. 10135163)

C. Potassium ferricyanide (JT. Baker, Cat No. 13746-66-2, Lot No. K27623)

D. Triton X-100 (Sigma, Cat No. T8787-250ML, Lot No. 089K01923)

E. Hemoglobin from bovine blood (Sigma, Cat No. H2500-1G, Lot No. 010K7618)

F. Dulbecco's Phosphate Buffer Saline (without $MgCl_2$ and $CaCl_2$, Sigma, Cat No. D5652-10L, Lot No. 081M8314)

3. Equipments

A. UV/Vis spectrometer (Thermo, BIOBATE3S, Equipment no. UVS-1)

B. Incubator (CI-60 62302, Equipment no. INB-8)

4. Preparation of cyanmethemoglobin reagents

Cyanmethemoglobin reagent is prepared by mixing 0.14g potassium phosphate, 0.05g potassium cyanide, 0.2g potassium ferricyanide, and 0.5mL Triton X-100 in 1L distilled water. The pH value of the reagent is adjusted to 7.0-7.4 and store at 4℃.

5. Preparation of blood substrates

A. Standard hemoglobin solution

Anti-coagulated rabbit blood is prepared and the anticoagulant is 0.13M citrate. The blood is stored at 4°C but no longer than 96 hr before using. The hemoglobin stock solution is prepared by dissolving 25mg hemoglobin standard in 25mL cyanmethemoglobin reagent with a final concentration of

1mg/mL. Six concentrations of standard hemoglobin solution (0.03, 0.07, 0.10, 0.30, 0.50, 0.70 mg/mL) are prepared and the absorbance is measured at 540 nm. A standard hemoglobin calibration curve is plotted using mg/mL on the y-axis and A540 on the x-axis and calibration coefficient (F) is the slope of this plot.

Calibration coefficient (F) = _____

B. Determination of plasma free hemoglobin (PFH)

3mL whole blood is centrifuged at 800×g for 15min. Mix 0.5mL of plasma and 0.5mL of cyanmethemoglobin solution, and the absorbance is measured at 540 nm after 15min. The total plasma free hemoglobin concentration is calculated as follow:

$PFH = A^{PFH} \times F \times 2$ = _____

The blood will be qualified and used for the study if the value of the PFH is less than 2mg/mL.

C. Total blood hemoglobin concentration (C) and diluted blood hemoglobin concentration (T)

20μL of whole blood is added to 5mL of cyanmethemoglobin reagent for 5min and then measurethe absorbance of the solution at 540 nm. Total blood hemoglobin concentration (C) is calculated as follow:

$C = A^{C} \times F \times 251$ = _____

Then the total blood hemoglobin concentration is adjusted to 10mg/mL with PBS (Mg^{2+} and Ca^{2+} free). The dilution blood 300μL is added to 4.5mL of reagent and then measure the absorbance of the solution at 540 nm to remain on the standard curve. This is a dilution factor of 16 and the diluted blood hemoglobin concentration (T) is calculated as follow:

$T = A^{T} \times F \times 16$ = _____

6. Preparation of Test Article and Control Article

A. Extract test:

a. Test Article

The test article is extracted by Dulbecco's Phosphate Buffer Saline (without $MgCl_2$ and $CaCl_2$) with a ratio of 0.2 g /1mL.

b. Control Articles

(i) Blank control: The extract buffer is as blank control

(ii) Positive control: Positive control is Buna N rubber extracted by Dulbecco's Phosphate Buffer Saline (without $MgCl_2$ and $CaCl_2$) with a ratio of 6 cm^2/1mL,

(iii) Negative control: Negative control (HDPE) is extracted by Dulbecco's Phosphate Buffer Saline (without $MgCl_2$ and $CaCl_2$) with a ratio of 6 cm^2/1mL.

c. Procedure of preparation:

(i) All of the controls have the same configuration as the test article which is extracted at 50±2°C for 72±2 hr with constant agitation at 150 rpm.

B. Direct contact:

a. Test Article

Weigh three 1.4 g test article pieces and then transfer each into individual tubes. Place 7.0 mL of no Mg^{2+} and Ca^{2+} PBS into each tube with a ratio of 0.2g/mL.

b. Control Articles

(i) Blank control: Place 7.0 mL of no Mg^{2+} and Ca^{2+} into each of three tubes to serve as the blank.

(ii) Positive control: Weigh three 1.4 g Buna N rubber pieces and then transfer each into individual tubes. Place 7.0 mL of no Mg^{2+} and Ca^{2+} PBS into each tube with a ratio of 0.2g/mL.

(iii) Negative control: Weigh three 1.4 g HDPE pieces and then transfer each into

individual tubes. Place 7.0 mL of no Mg^{2+} and Ca^{2+} PBS into each tube with a ratio of 0.2g/mL.

C. Hemolysis test

a. Each 1 mL of diluted blood is added into 7 mL of the resultant extractions / above tube and incubated at least 3 hr ± 10 min at 37±1°C

b. Treatments of the diluted blood with the extracts/the test articles and controls are performed in triplicates.

c. Invert each tube gently every 30 min during incubation and transfer the fluid to another tube and centrifuge at 800 g for 15 min after 3 hr incubation.

d. 1mL supernatant is added to the same volume of cyanmethemoglobin reagent and measure the absorbance of the solution at 540nm.

7. Calculation method

A. The hemoglobin concentration of test article (S) and the hemoglobin concentration of blank (B) are calculated **as follow:**

Test article (S) = $A^S \times F \times 2$ = _____

Blank (B) = $A^B \times F \times 2$ = _____

B. The estimate percentage of hemolysis is calculated as follow:

$$\% \text{ hemolysis} = \frac{\text{Concentration of hemoglobin released in supernatant} \times 100\%}{\text{Total hemoglobin concentration}} = \_\_\_\_$$

C. The percentage of hemolysis corrected for the blank is calculated as follow:

$$\text{Blank corrected \% hemolysis} = \frac{(A^S - A^B) \times 100\%}{(A^T - A^B)} = \_\_\_\_$$

8. Quality Criteria

A. The blood is stored at 4±2°C and used within 48hrs.

B. Equal quantities of blood from each rabbit should be pooled.

C. The percentage of corrected hemolysis for positive control should be more than 5%

D. The percentage of corrected hemolysis for negative control should be less than 2%

DATA MANAGEMNT

The qualitative data were scored using "Hemolytic index and hemolytic grade" (Table 1). The individual score represents the average of triplicates. Mean score was the average of the qualitative scores.

RESULTS

(1) Extract test:

A. Standard hemoglobin solution

Calibration coefficient (F) = 2.31

B. Determination of plasma free hemoglobin (PFH)

$PFH = A^{PFH} \times F \times 2 = 0.73$

C. Total blood hemoglobin concentration (C) and diluted blood hemoglobin concentration (T)

$C = A^{C} \times F \times 251 = 208$

$T = A^{T} \times F \times 13.5 = 9.18$

D. Hemolysis test of extract (S)

Blank (B) = 0.008

The test article extract (S) = 0.012

Negative control (S) = 0.008

Positive control (S) = 1.06

E. Estimate percentage of hemolysis:

% hemolysis of the test article = 1.07

% hemolysis of negative control = 0.669

% hemolysis of positive control = 92.1

F. Percentage of hemolysis corrected for the blank:

Blank corrected % hemolysis of the test article = 0.400

Blank corrected % hemolysis of negative control = 0.000

Blank corrected % hemolysis of Positive control = 92.1

G. Table

Test article	S value	%hemolysis	Blank corrected % hemolysis	Hemolytic Grade
UB70737	0.012	1.07	0.400	Non-hemolytic
Negative	0.008	0.669	0.000	Non-hemolytic
Positive	1.06	92.1	92.1	Hemolytic

(2) Direct contact:

A. Standard hemoglobin solution

Calibration coefficient (F) = 2.31

B. Determination of plasma free hemoglobin (PFH)

$PFH = A^{PFH} \times F \times 2 = 0.73$

C. Total blood hemoglobin concentration (C) and diluted blood hemoglobin concentration (T)

$C = A^{C} \times F \times 251 = 208$

$T = A^{T} \times F \times 13.5 = 9.18$

D. Hemolysis test of the test article (S)

Blank (B) = 0.017

The test article (S) = 0.002

Negative control (S) = 0.002

Positive control (S) = 1.02

E. Estimate percentage of hemolysis:

% hemolysis of the test article = 0.134

% hemolysis of negative control = 0.134

% hemolysis of positive control = 89.2

F. Percentage of hemolysis corrected for the blank:

Blank corrected % hemolysis of the test article = 0.000

Blank corrected % hemolysis of negative control = 0.000

Blank corrected % hemolysis of Positive control = 89.0

G. Table

Test article	S value	%hemolysis	Blank corrected % hemolysis	Hemolytic Grade
UB70737	0.002	0.134	0.000	Non-hemolytic
Negative	0.002	0.134	0.000	Non-hemolytic
Positive	1.02	89.2	89.0	Hemolytic

CONCLUSION

The percent hemolysis of the test article "Transducer Protector" extract was 1.07 % in extract test; the percent hemolysis of the test article was 0.134 % in direct contact test. Base on the hemolytic index, the "Transducer Protector" was non-hemolytic according to ISO10993-4 and ASTM F756-08.

DEVIATIONS AND INVESTIGATIONS

There was no deviation and investigation during the test period of this study.

PROTOCOL AMENDMENTS

There was no protocol amendment during the test period of this study.

REFERENCES

1. Good Laboratory Practice for Nonclinical Laboratory Studies. Title 21 of the U.S. Code of Federal Regulations, Part 58 (1997) United States Food and Drug Administration.
2. ISO 10993 (2006) Biological evaluation of medical device－Part 4: Selection of tests for interactions with blood.
3. ASTM F756-08: Standard Practice for Assessment of Hemolytic Properties of Materials.
4. ASTM F619-03 (Reapproved 2008): Standard Practice for Extraction of Medical Plastics.
5. Malinauskas, R. A. Plasma hemoglobin measurement techniques for the *in vitro* evaluation of blood damage caused by medical devices, Artif. Organs, 21, 1997, p. 1255-1267
6. Henry, J. B. Hematology and Ckoagulation, In: Clinical Diagnosis & Management By Laboratory Methods, 18th edn., W.B. Saunders Co., Philadelphis, PA, USA, 556-603, 1991.
7. ISO 10993 (2012) Biological evaluation of medical devices-Part 12：Sample preparation and reference materials
8. Current OECD Principles of Good Laboratory Practice (Organization for Economic Cooperation and Development, Paris, ENV/MC/CHEM (98) 17).
9. TESP-UB-1012 The hemolysis testing for medical device Version 1.4.

TABLE

Table 1 - Hemolytic Index and Hemolytic Grade

Blank corrected % hemolysis	Hemolytic grade
<2	Non-hemolytic
2-5	Slightly hemolytic
>5	Hemolytic

TEST ARTICLE PHOTO