TEST REPORT

STUDY REPORT NUMBER: MB-**TC-01**-E-16-10-A2

(Replacement to study report number MB-TC-01-E-16-10-A1)

Study Completion Date: 16 JUN 2011

Title: TEST FOR CYTOTOXICITY

(MEM ELUTION ASSAY)

(Ultrasound Gel)

Study Sponsor	**Testing Facility**
TELE-PAPER (M) Sdn Bhd **Lot 2C, Jalan Keluli 15/16,** **Section 15,** **40200 Shah Alam,** **Selangor, Malaysia**	**Makmal Bioserasi** INBIOSIS Universiti Kebangsaan Malaysia 43600 UKM Bangi Selangor Malaysia.

Study Director : Assoc Prof Dr Md Anuar Osman

Quality Assurance Personnel : Nurdiana Ishak

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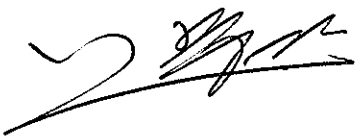
- This report contains 11 pages.
- This test report concerns only the product being tested.

TEST FOR CYTOTOXICITY (MEM ELUTION ASSAY)

Study Director	**Signature**
Assoc. Prof. Dr Md Anuar Osman *DVM (Pak), M. Sc (W. Aust), PhD (Murdoch)*	
	Date: 16/6/2011
Sponsor	**Signature**
TELE-PAPER (M) SDN BHD (296787-W) Lot 2C, Jalan Keluli 15/16, Section 15, 40200 Shah Alam Selangor Darul Ehsan TEL : (603) 5122 8660 FAX : (603) 5122 8660	
	Date: 20/6/2011

TEST REPORT – MEM Elution Assay (ISO)

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SUMMARY

MEM Elution Assay

Protocol Reference : PR-TC-01

Method Used : ISO 10993-5:2009(E)

Study Completion Date : 16 Jun 2011

Study Reference Number : TC-01-22-10

Study Report Number : MB-TC-01-E-16-10-A2
(Replacement to study report number MB-TC-01-E-16-10-A1)

Job Number : E-16-10

Test Material : SM Gel (Ultrasound Gel)

Lot / Batch Number : N/A

1. OBJECTIVE

To assess the cytotoxic potential of a test material solution of SM Gel (Ultrasound Gel) using a mammalian cell line as the target cells.

2. EXPERIMENTAL PROCEDURE

Cell culture: American Type Culture Collection L-929 mouse subcutaneous connective tissue fibroblast cells (*Mus musculus*, NCTC clone 929, CCL-1^{TM}), with passage number 6.

Preparation of cell culture	:	3 May 2010
Preparation of test material	:	4 May 2010
Treatment	:	4 May 2010
End of test	:	5 May 2010

Study: Cytotoxicity assessment of endogenous and extraneous substances present in the test material.

The test was carried out using SM Gel (Ultrasound Gel) as a test material. A confluent monolayer of cultured cells was treated with varying concentrations of test material solution. Cytotoxicity was examined by microscopic assessment of changes in the monolayer and cell death. The degree of cytotoxicity was graded according to USP 26 Biological Reactivity Tests, *In Vitro* <87> as follows:

Grade	Reactivity	Conditions of all cultures
0	**None**	**monolayer complete, no cell lysis**
1	**Slight**	**cell death not more than 20%**
2	**Mild**	**cell death not more than 50%**
3	**Moderate**	**cell death not more than 70%**
4	**Severe**	**complete destruction of the cell monolayer**

3. RESULTS

Table 1: Reactivity grades of test material solution of SM Gel (Ultrasound Gel) and controls.

			Cytotoxicity Grade	
	Concentration	Wells	4 May 2010	5 May 2010
Sky Gel (Ultrasound Gel)	100%	A – B – C	0 – 0 – 0	2 – 2 – 2
	50%	A – B – C	0 – 0 – 0	0 – 0 – 0
	25%	A – B – C	0 – 0 – 0	0 – 0 – 0
	12.5%	A – B – C	0 – 0 – 0	0 – 0 – 0
	6.25%	A – B – C	0 – 0 – 0	0 – 0 – 0
	3.125%	A – B – C	0 – 0 – 0	0 – 0 – 0
Negative control		A – B – C	0 – 0 – 0	0 – 0 – 0
Positive control		A – B – C	0 – 0 – 0	4 – 4 – 4

The test material solution of **SM Gel (Ultrasound Gel)** produced a mild cytotoxic effect (grade 2) at 100% concentration. No cytotoxic effects were observed at 50%, 25%, 12.5%, 6.25% and 3.125% concentrations. Both the negative and positive control items performed as anticipated.

4. CONCLUSION

The test material solution of **SM Gel (Ultrasound Gel)** produced a mild cytotoxic effect (grade 2) at 100% solution concentration under the condition of this test.

TEST REPORT

1.0 SPONSOR OF TEST MATERIAL

1.1	**Name**	:	TELE-PAPER (M) Sdn Bhd
1.2	**Address**	:	Lot 2C, Jalan Keluli 15/16, Section 15, 40200 Shah Alam, Selangor
1.3	**Study report number**	:	MB-TC-01-E-16-10-A2 *(Replacement to study report number MB-TC-01-E-16-10-A1)*
1.4	**Job number**	:	E-16-10

2.0 DETAILS OF TEST MATERIAL

2.1	**Name**	:	SM Gel (Ultrasound Gel)
2.2	**Test material reference**	:	MD16-E0410
2.3	**Study reference number**	:	TC-01-22-10
2.4	**Lot / Batch number**	:	N/A
2.5	**Date received**	:	16 April 2010
2.6	**Expiry date**	:	N/A
2.7	**Appearance**	:	Gel
2.8	**Colour**	:	Blue
2.9	**Quantity**	:	2 packets
2.10	**Storage**	:	Refrigerated
2.11	**Dates of test**	:	3 May – 5 May 2010
2.12	**Date of completion**	:	5 May 2010

3.0 LABORATORY FACILITY

3.1	**Name**	:	Makmal Bioserasi
3.2	**Address**	:	INBIOSIS, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor, Malaysia.

3.3 Project staff

3.3.1 Study Director
Assoc. Prof. Dr Md. Anuar Osman
DVM (Pak), M.Sc (W. Aust), PhD (Murdoch)

3.3.2 Study Personnel
Zahidah Ismail, B.Sc (Hons)

3.3.3 Quality Assurance Personnel
Nurdiana Ishak, B.Biomed.Sc

3.4 Address of correspondence

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Tel : 03 8921 4280
Fax : 03 8921 4281

c/o
Assoc Prof Dr Md Anuar Osman

3.5 Study timetable

3.5.1	Preparation of cell culture	:	3 May 2010
3.5.2	Preparation of test material	:	4 May 2010
3.5.3	Treatment	:	4 May 2010
3.5.4	End of test	:	5 May 2010

3.6 Reagents and Apparatus

3.6.1 Purified water. Water used in the study was produced by reverse osmosis (ELGA).

3.6.2 Growth medium. Eagle's Minimum Essential Medium (EMEM) with Earle's salt (Flowlab) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (PAA), non-essential amino acid (Hyclone), 100 IU penicillin and 100 µg/ml streptomycin (Hyclone).

3.6.3 Phosphate buffered saline solution (calcium and magnesium free) (SIGMA).

3.6.4 Trypsin-EDTA 0.025% (SIGMA).

3.6.5 Trypan blue (0.4%) solution.

3.6.6 Sterile plastic containers of tissue culture grade: 25 cm^2 tissue culture grade flask (NUNC), 75 cm^2 tissue culture flask (NUNC), 24-well tissue culture plate (NUNC), 15 ml conical tube (Falcon) and 50 ml conical tubes (Falcon).

3.6.7 Haemocytometer counting chamber (Hirschmann Laborgerate).

3.6.8 Single and multichannel pipettor (Treff Lab).

3.6.9 Sterile 0.22 µm syringe filter unit (Sartorius).

3.6.10 Inverted microscope with phase contrast (Nikon Eclipse TS100).

3.6.11 Carbon dioxide heat-coil-jacketed incubator (RS Biotech, model Galaxy R).

3.6.12 Biological safety cabinet Class II (Bioair Instruments, model Aura 2000).

4.0 TEST METHOD

4.1 Name of test: MEM Elution Assay

The degree of cytotoxicity in a mammalian cell culture in response to the test material solution was determined (according to ISO 10993-5:2009(E). Biological evaluation of medical devices – Part 5: Test for *in vitro* Cytotoxicity). Sky Gel (Ultrasound Gel) was dissolved in growth medium and was diluted to working concentration. Positive control (zinc sulfate) was used in the study to verify the proper functioning of the test system. The test material was tested in triplicate at 100%, 50%, 25%, 12.5%, 6.25% and 3.125% concentrations. Cultures were incubated at 37°C in a carbon dioxide incubator for 24 hours and examined microscopically for signs of monolayer changes and cell death.

4.2 Objective

To assess the cytotoxic potential of a test material solution of Sky Gel (Ultrasound Gel) using a mammalian cell line as the target cells.

4.3 Significance and rationale

This method is useful for assessing the cytotoxic potential of new materials and formulations and as part of a quality control program for an established or new medical device and its components. Assessment of cytotoxicity provides useful information in predicting the potential clinical applications in the human. Cell culture methods have shown good correlation with animal assays and are frequently more sensitive to cytotoxic agents.

4.4 Cell culture

American Type Culture Collection L-929 mouse subcutaneous connective tissue fibroblast cells (*Mus musculus*, NCTC clone 929, CCL-1™), with passage 6.

4.5 Test procedure

The procedure was divided into four stages as follows:

a) Cells were grown and maintained as healthy monolayer cultures.
b) Test material preparation.
c) Cultures were subjected to treatment with the test material.
d) Assessment of the effects of test material solution on cell morphology.

4.5.1 Cell culture maintenance

L-929 cells were grown in tissue culture flasks using EMEM as the growth medium. Cultures were examined daily to ensure they remain healthy. Any changes in morphology (such as increase in size) or their adhesive properties were noted.

4.5.2 Preparation of cell culture

Cells were grown as a monolayer at 37°C in a humidified atmosphere of 5% carbon dioxide and 95% air. The confluent monolayer was removed by trypsinisation and the number of viable cells was calculated. Cells were seeded into a 24-well plate and incubated at 37°C for at least 12 hours or until attaining 80% confluency.

4.5.3 Preparation of test material

The test material **(SM Gel (Ultrasound Gel))** was dissolved in EMEM growth medium at 200mg/ml concentration and was diluted with EMEM growth medium to **2mg/ml** working concentration. The test material solution was sterilized via membrane filtration.

4.5.4 Exposure of test material to cell culture

The test material was tested at 100%, 50%, 25%, 12.5%, 6.25% and 3.125% concentrations. Growth medium from each well of a 24-well plate containing healthy culture was replaced with 1 ml of the test material solution. The cultures were then incubated for 24 hours at 37°C in a humidified atmosphere of 5% carbon dioxide and 95% air.

4.5.5 Assessment of results

A qualitative method was used to determine the cytotoxic effects. The condition of cells in each well was examined microscopically and the degree of cytotoxicity was graded using the reactivity scoring method of USP 26.

Grade	Reactivity	Conditions of all cultures
0	**None**	**monolayer complete, no cell lysis**
1	**Slight**	**cell death not more than 20%**
2	**Mild**	**cell death not more than 50%**
3	**Moderate**	**cell death not more than 70%**
4	**Severe**	**complete destruction of the cell monolayer**

5.0 NUMBER OF TEST MATERIAL / REPLICATES

Triplicate.

6.0 CONTROLS

Growth medium (negative) and zinc sulfate solution (positive).

7.0 QUALITY ASSURANCE

The final report was audited in agreement with the raw data record and for compliance with the protocol and Standard Operating Procedures of Makmal Bioserasi. Dates of inspections and audits performed during the study and the dates of reporting of the inspection and audit findings to the Study Director are presented in the Quality Assurance Statement.

8.0 DEVIATIONS FROM APPROVED PROTOCOL

None.

9.0 RECORDS TO BE MAINTAINED

A copy of this signed report, together with the protocol and all raw data generated during the study are retained in Makmal Bioserasi archive.

10.0 DATES OF TEST PROCEDURE

Preparation of cell culture	:	3 May 2010
Preparation of test material	:	4 May 2010
Treatment	:	4 May 2010
End of test	:	5 May 2010

11.0 RESULTS

Table 1: Reactivity grades of test material solution of SM Gel (Ultrasound Gel) and controls.

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	Concentration	Wells	4 May 2010	5 May 2010
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	6.25%	A – B – C	0 – 0 – 0	0 – 0 – 0
	3.125%	A – B – C	0 – 0 – 0	0 – 0 – 0
Negative control		A – B – C	0 – 0 – 0	0 – 0 – 0
Positive control		A – B – C	0 – 0 – 0	4 – 4 – 4

The test material solution of **SM Gel (Ultrasound Gel)** produced a mild cytotoxic effect (grade 2) at 100%. No cytotoxic effects were observed at 50%, 25%, 12.5%, 6.25% and 3.125% concentrations. Both the negative and positive control items performed as anticipated.

12.0 CONCLUSION

The test material solution of **SM Gel (Ultrasound Gel)** produced a mild cytotoxic effect (grade 2) at 100% solution concentration under the condition of this test.

13.0 REFERENCES

13.1 ISO 10993-5:2009(E). Biological evaluation of medical devices – Part 5: Test for *in vitro* cytotoxicity.

13.2 ISO 10993-12:2007(E). Biological evaluation of medical devices – Part 12: Sample preparation and reference materials.

13.3 US Pharmacopeia (USP) 26. 2003. Biological Reactivity Tests, *In Vitro* <87>.

14.0 VERIFICATION

I the undersigned declare that the methods, results and data contained in this report faithfully reflect the procedures used and the raw data collected during the study.

Assoc. Prof. Dr Md. Anuar Osman, PhD
Study Director

16/6/2011
Date

15.0 QUALITY ASSURANCE INSPECTIONS STATEMENT

This study has been inspected by the Quality Assurance Personnel, and the findings have been reported to Study Director on the following dates:

Procedures Inspected	Inspected Dates	Reported Dates
a. Records & Report	13 May 2010	13 May 2010

Nurdiana Ishak
Quality Assurance Personnel

16 Jun 2011
Date