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STUDY REPORT

BIOLOGICAL, STRUCTURAL AND STABILITY STUDIES ON FABRIC MATERIAL USED FOR ROTATOR CUFF REPAIR DEVICE FOR SHOULDER RECONSTRUCTION

Report No and Date: TRAAS046.001 dated 18th December 2013

Study Director: Muraleedharan CV, Engineer G

Report Issued to :

The South India Textile Research Association
Coimbatore

1. IDENTIFICATION OF THE STUDY

DTL AAS 046: BIOLOGICAL, STRUCTURAL AND STABILITY STUDIES ON FABRIC MATERIAL USED FOR ROTARY CUFF REPAIR DEVICE FORSHOULDER RECONSTRUCTION

2. SUMMARY OF THE STUDY

Biological, structural and stability studies were done on the polyester fabric material for the rotator cuff repair device. The tests were done on the basis of a protocol (DTLAAS046) mutually agreed by Ms. SITRA and SCTIMST.

3. STUDY PROTOCOL

3.1 Objective:

To evaluate the suitability of the polyester fabric material for use as rotator cuff repair device.

3.2 Study Design :

Mechanical Tests

The tests will be done as per the following standards

- ISO 13934-1:Textiles-Tensile Properties of Fabrics
- ISO 7198: Cardiovascular device: tubular grafts

Physico Chemical Tests

Material characterization is the crucial first step in the biological evaluation process. The extend of chemical characterization depends on what pre-clinical and clinical safety and toxicological data exist, and on the nature and duration of body contact with the medical device, but as a minimum the characterization shall address the constituent chemicals of the device and constituent residual process aids or additives used in its manufacture. The following physico-chemical tests would be done

1. Material Identification- By IR Spectroscopy
2. Trace Element Analysis
3. Thermogravimetry
4. Differential Thermal Analysis

Stability Studies

Accelerated ageing is the storing of materials at elevated temperature and / or other intensified environmental conditions in order to simulate real time ageing in a smaller duration of time. The increased temperature contributes towards a faster kinetics of material degradation. It is a proven fact that the ageing of medical device and biomaterials (especially polymer based ones) can be accelerated to a two times pace by increasing the storage temperature by 10°C, by what is known as Q_{10} analysis. (A Q_{10} analysis involves testing of the package integrity at various temperatures and defining the difference in degradation rate for a 10°C rise in temperature). Based on the Q_{10} analysis on various types of materials, it is now common practice to use a conservative value for $Q_{10} = 2$, for assessing the necessary period of accelerated ageing test for qualifying the materials for a specific shelf life period.

Accelerated ageing factor is

$$AAF = Q_{10}^{(Te - Ta) / 10}$$

where, Ta = Ambient storage temperature

Te = Accelerated ageing test temperature

$Q_{10} = 2$ (every 10°C rise doubles package degradation rate).

For the test device under consideration, following ageing conditions are arrived at

(a) Storage temperature: 30 ±2 °C

(b) Duration of simulated ageing: Five years

(c) Mean ageing temperature: 60°C (The ageing temperature is chosen to ensure that the material does not undergo any chemical transformations during the ageing process and shall not exceed 65°C for polymeric materials)

$AAF = 2^{(60 - 30) / 10} = 8$

Based on the ageing factor, the ageing duration is estimated as (5*360)/8 =225` days.

(1) Accelerated ageing temperature: 60 °C ± 3 °C

(2) Relative humidity of the chamber: 60 % ± 20% RH

(3) Ageing duration: 225 days.

The following tests will be done after ageing

1. Thermo-gravimetry
2. Differential Thermal Analysis
3. Tensile Strength and elongation at break
4. Burst Strength

Biological Characterization

Biological Characterization will be done as per ISO 10993 series standards. The ISO 10993 set entails a series of standards for evaluating the biocompatibility of a medical device prior to a clinical study. The following biological tests would be done

1. In Vitro Cytotoxicity
   - Test on extract
   - Direct contact
2. Acute Systemic Toxicity
3. Intracutaneous Reactivity
4. Intramuscular Implantation
5. Blood Compatibility
   - Hemolysis
   - Coagulation Profile
   - Response to whole blood
   - Complement Activation
6. Sensitization

3.3 Methodology :

Preparation of Samples

The samples were prepared as per the following procedure

a) Cutting the samples to required sizes for each test

b) Heat setting to shape the fabric

c) Ultrasonic cleaning in 1% Extran (Neutral) in DI water for ten minutes, followed by 10 rinses in DI water, with every third in ultrasonic cleaner for five minutes

d) Drying at 60°C for 3 hours

e) Isopropyl alcohol extraction using a soxhlet apparatus for 10 cycles

f) Drying at 60°C for 6 hours

g) Water extraction at 60°C for 18 cycles, each cycles for 20 minutes with DI water change between each cycle

h) Drying at 60°C for 6 hours (Validation with repeat weighing)

i) Packaging in Tyvek bags for sterilization

j) Sterilization using ETO, 600mg/L concentration, 90 minutes exposure, 60% RH in environment (noncondensing), six cycles of aeration with 0.22 micron filtered air

k) Quarantine for one week for EO to ensure residual reduction before all biological studies

The details of the tests conducted are provided in table 1 below

Table 1(a) : **Mechanical Tests**

No	Name of Test	Standard followed	Number of Samples / test
1	Burst Strength	ISO 7198	Five
2	Tensile Strength and Elongation at break	ISO 13934-1	Five
3	Suture Retention	ISO7198	Five
4	Permeability	ISO7198	Five

Table 1 (b): **Physico Chemical Studies**

Sl No	Name of Test	Standard followed	Number of Samples / test
1	Material Identification	ASTM E1252	One
2	Trace Element Analysis (Heavy Metal Identification)	ICP-AES	One
3	Thermo-gravimetry	ASTM E1131	One
4	Differential Thermal Analysis	ASTM E537	One

Table 1 (C): Stability Studies (Tests done after Ageing)

Sl No	Name of Test	Standard followed	Number of Samples / test
1	Thermo-gravimetry	ASTM E1131	One
2	Differential Thermal Analysis	ASTM E537	One
3	Burst Strength	ISO7198	Five
4	Tensile Strength and Elongation at break	ISO 13934-1	Five

Table 1(d): Biological Characterization Studies

Sl No	Name of Test	Standard followed	Number of Samples / test
1	In Vitro Cytotoxicity	ISO 10993-5:2009	1 set
2	Acute Systemic Toxicity	ISO 10993-11:2006	1 set
3	Intracutaneous Reactivity	ISO 10993-10:2010	1 set
4	Intramuscular Implantation	ISO 10993-6:2007	1 set
5	Blood Compatibility	ISO-10993-4:2002 /AMD 1:2006	1 set
6	Sensitization	ISO 10993-10:2010	1 set

3.4 Test Equipment :

Accelerated Ageing

Name of the Equipment : Environmental Chamber

Equipment ID No : EQDTL010

Accuracy specifications :

Humidity distribution: ± 3%

Temperature distribution: ±0.5°C

Mechanical Testing

Name of Equipment: Instron UTM model 3345

Load Cell: 5 kN - Model No 66515

Software: Blue Hill Version2.22.773

Test Grip: 5KN- Model No.2710-105

3.5 Test Samples :

Sample name: Polyester fabric for rotator cuff repair device

Sample details: The samples received from the client was cut to size according to the requirements of the individual tests and renumbered from DTLAAS046.001 to DTLAAS046.077for internal tracking

4. OBSERVATIONS AND RESULTS

The samples supplied were aged for periods as mentioned in the schedule provided. The profile of the temperature / humidity variation in the accelerated ageing chamber is provided in fig 1. The consolidated results for the different tests are provided in succeeding subsections.

MECHANICAL CHARACTERIZATION

Burst strength Test: In the burst strength test an area of the sample is clamped over an orifice by means of a flat annular clamp ring and a cylindrical probe with a hemispherical head is traversed through the specimen until it ruptures. The test measures the force required for rupture. The burst strength test was conducted on samples without any ageing and on samples which had undergone accelerated ageing. See table 2 for consolidated results (Refer TR AAS046.002 (without ageing) and TR AAS046.006 (aged samples) for details). The results from the burst strength studies indicate that there is no reduction in strength after the ageing process. The marginal increase in the burst strength observed for the aged samples is well within the standard deviation and is not statistically significant (Based on student t test, p=0.35)

Sl. No	Before Ageing		After Ageing	
	Maximum Load (N)	Burst Strength (N/mm²)	Maximum Load (N)	Burst Strength (N/mm²)
1	218.1	2.28	222.9	2.33
2	209.1	2.19	201.9	2.11
3	252.4	2.64	187.6	1.96
4	164.2	1.72	199.4	2.09
5	181.7	1.90	273.4	2.86
6	146.1	1.53	204.2	2.13
Mean		2.04		2.25
Standard Deviation		0.41		0.32

Table 2: Burst Strength Characteristics, before and after ageing

Tensile Strength and Elongation at Break: In the tensile strength the fabric material was stretched at a constant rate of extension of 50mm/min using a Universal Testing Machine tills the material breaks. The tensile strength test was conducted on samples without any ageing and on samples which had undergone accelerated ageing See tables 3 for consolidated results

(Refer TR AAS046.003 (without ageing) and TR AAS046.007 (aged samples) for details). The results from the tensile strength studies indicate that there is a marginal decrease in the average tensile load at break (~20%, which was statistically significant, p=0.03) indicating marginal reduction in strength after the ageing process. But there was no change in the elongation at break (statistically not significant, p=0.94).

Sl. No	Before Ageing		After Ageing	
	Tensile Load at Break (N)	Elongation at Break (%)	Tensile Load at Break (N)	Elongation at Break (%)
1	571.2	82.0	671.3	69.0
2	702.5	111.7	432.2	115.0
3	654.5	107.3	553.4	71.2
4	678.5	84.5	394.5	109.3
5	672.3	69.5	533.3	86.3
Mean	655.9	90.9	517.0	90.2
SD	50.3	17.9	109.2	21.3

Table 3: Tensile Strength Characteristics, before and after ageing

Suture Retention Test : The test measures the force necessary to pull a suture from the fabric or cause the wall of the fabric to fail under tensile extension. The material was tested for three standard sizes of sutures(USP 0,USP 2-0 and USP 3-0) .See table 4 for consolidated results (Refer TR AAS046.004 for details). The values are well within the suture retention requirements observed for medical textiles.

Srl No	Suture Retention Force (Newton)		
	USP 0	USP 2-0	USP 3 -0
1	38.06	28.67	17.55
2	30.87	73.08	27.80
3	24.52	31.73	28.18
4	41.00	52.46	27.05
5	49.01	14.14	40.04

Mean	34.94
Standard Dev	14.88

Table 4: Suture Retention Characteristics

Water Permeability Test: The test is intended to measure the rate of flow of water through a given area of the sample prosthesis under a given hydrostatic pressure. See table 5 for consolidated results (Refer TR AAS046.005 for details). Water permeability is found to be repeatable at different locations of the fabric indicating the relative consistencey in the fabric architecture and structure. The values are close to that observed for knitted vascular grafts. It is known that good structural posoristy improves tissue incorporation and there by better healing.

Srl No	Flow Rate (Q) [ml/min]	Water Permeability(Q/A) [ml/cm2/min]
1	1239	5208
2	1003	4217
3	1115	4687
4	1010	4244
5	1171	4923
	Mean	4660
	SD	430

Table 5: Suture Retention Characteristics

PHYSICO CHEMICAL CHARACTERIZATION

Material Identification: Material Identification was done using Fourier Transform Infrared Spectroscopy(FTIR Spectroscopy). FTIR analysis gives a wavelength versus absorption spectrum and by matching the IR spectrum of an unknown material with that of a known material, proof of identity of the material can be established. The FTIR spectrum for the material was recorded and found to match that of PET. (Refer DPL/FTIR/16/13 for details)

Trace Element Analysis: Presence of heavy metal impurities in the fabric material was investigated using trace element analysis. Presence of four heavy metals Arsenic, Cadmium, Mercury and Lead was investigated. Trace Element Estimation was done using Optical Emission Spectroscopy with inductively coupled plasma. The test samples were digested in

concentrated HNO_3 + concentrated $HClO_4$ mixture and then extracted using de-ionized water. See table 6 for consolidated results (Refer TRICP 002.Y13 for details)

Analyte Element	Amount of analyte element in the sample (ppm)	BDL(ppm)
As118.979	3.52	NA
Cd228.802	BDL	0.003
Hg253.652	BDL	0.061
Pb220.353	BDL	0.042

*BDL- Below Detection Limit

Table 6: Results from trace element analysis

Thermogravimetry: The thermogravimetry test is done to determine the mass loss of the material as a function of temperature. TGA is effective for quantitative analysis of thermal reactions that are accompanied by mass changes, such as evaporation, decomposition, gas absorption, desorption and dehydration. The material was tested up to a maximum temperature of 600 deg Celsius. The mass (%) versus temperature plot (thermogram) was recorded. The thermogravimetry test was conducted on samples with no ageing and samples that had undergone accelerated ageing. See attached reports TRLPA/TGA343.Y13(no ageing) and TRLPA/TGA360.Y13(aged sample) for details. No specific changes were observed in the key characteristics of the aged and unaged materials, indicating minimal degradation during the ageing process.

Differential Thermal Analysis: DTA measures the temperature difference between a sample and a reference material as a function of temperature as they are heated or cooled or kept at a constant temperature (isothermal).DTA provides vital information of the materials regarding their endothermic and exothermic behavior at high temperatures. The material was tested up to a maximum temperature of 600°C. The thermogram was recorded during the analysis. DTA was conducted on samples with no ageing and samples that had undergone accelerated ageing. See attached reports TRLPA/DTA148.Y13 (no ageing) and TRLPA/DTA154.Y13 (aged

sample) for details. No specific changes were observed in the key characteristics of the aged and unaged materials, indicating minimal degradation during the ageing process.

BIOLOGICAL CHARACTERIZATION

In Vitro Cytotoxicity: Direct Contact and Test on Extract was done to evaluate cytotoxicity as per ISO 10993-5. In the direct contact test the material showed non-reactivity to fibroblast cells after 24 hours of contact. In the test on extract the extract was prepared by incubating 3 cm^2 of test material on 1ml physiological saline at 50 ±2°C at 72±2hours.In the test on extract the material showed non-reactivity to fibroblast after 24 hours of contact.

Acute Systemic Toxicity: Acute Systemic Toxicity test was done using saline extract and cotton seed oil extract of the material.

Saline Extract: The study was designed to evaluate the systemic response of mice following intravenous injection of physiological saline extract of the test material, polymer, DTLAAS046-021. The study was conducted in accordance with 'ISO 10993-11: 2006 (E), Annex A.8, Test for systemic toxicity: Acute systemic toxicity test: Acute intravenous application and USP 34/NF 29: 2011, systemic injection test and in compliance with OECD principles of GLP. In this study there were 10 mice for the extract (5 for test and 5 for control). The body weight range of the animals was 17-23g. The physiological saline (PS) extract of the test material (DTLAAS046-021) and control (PS alone) was injected to the mice and observed immediately after injection and at 4h, 24h, 48h and 72h for the evidence of abnormalities such as any clinical signs, loss in body weight or death.

The result of the study *indicated* that the physiological saline extract of the test material and control injected animals did not show any abnormalities or loss in body weight during the observation period and confirmed that the physiological saline extract of the test material is non toxic at the laboratory conditions simulated. Hence, the physiological saline extract of the test material, polymer, DTLAAS046-021 (WITOXD70.Y13) meet the requirements of the test as per ISO 10993-11:2006(E), Annex A.8, Test for systemic toxicity: Acute systemic toxicity test: Acute intravenous application and USP 34/NF 29:2011, Systemic injection test.

Cotton Seed Oil Extract: The study was designed to evaluate the systemic response of mice following intraperitoneal injection of cotton seed oil extract of the test material, polymer, DTLAAS046-021.,. The study was conducted in accordance with 'ISO 10993-11:2006(E), Annex A.7, Test for systemic toxicity: Acute systemic toxicity test: Acute intra-peritoneal application and USP 34/NF 29:2011, Systemic injection test and in compliance with OECD principles of GLP. In this study there were 10 mice for the extract (5 for test and 5 for control). The body weight range of the animals was 17-23g. The cotton seed oil (CSO) extract of the test material (DTLAAS046-021) and control (CSO alone) was injected to the mice and observed immediately after injection and at 4h, 24h, 48h and 72h for the evidence of abnormalities such as any clinical signs, loss in body weight or death. The result of the study indicated that the cotton seed oil extract of the material and control injected animals did not show any abnormalities or loss in body weight during the observation period and confirmed that the cotton seed oil extract of the test material is non toxic at the laboratory conditions simulated.

Intracutaneous Reactivity: The study was designed to determine the irritation potential of the physiological saline and cotton seed oil extracts of the test material, polymer, DTLAAS046-025, following intradermal injection in albino Rabbits. The study was conducted in accordance with 'ISO 10993-10:2010(E), Biological evaluation of medical devices-part 10: Test for irritation and skin sensitization, Clause 6.4: Animal Intracutaneous (intradermal) reactivity test and USP 34/NF 29:2011 and in compliance with OECD principles of GLP. In this study there were 3 Rabbits for each material. Healthy adult animals not less than 2kg and not previously used were used for the test. The physiological saline (PS) and cotton seed oil (CSO) extracts of the test material was aseptically injected into 5 sites (0.2mL/site) on the upper left hand side and right hand side of 3 Rabbits. The physiological saline (control) alone and cotton seed oil (control) alone were injected into 5 sites on the lower left hand side and lower right hand side of the same Rabbits. The grading of erythema and oedema of test and control sites of all animals at 24, 48 and 72h were recorded as per ISO 10993-10:2010(E).The results indicated that the physiological saline and cotton seed oil extracts of the test material, DTLAAS046-025 (WITOX D73.Y13) produced a total mean score of '0' in physiological saline extract and '0.26' in Cotton seed oil extract following intradermal injection. Hence, the test material, Polymer, DTLAAS046-025 meets the requirements of the test as per ISO 10993-10:2010(E) – Biological evaluation of

medical devices: Part 10: Tests for irritation and skin sensitization: Clause 6.4: Animal Intracutaneous (Intradermal) reactivity test.

Intramuscular Implantation: This study was designed to evaluate the systemic response of muscle tissue following implantation of the test material, polymer, DTLAAS046-029The study was conducted in accordance with 'ISO 10993-11:2007(E): Biological evaluation of medical devices- Part 6: Test for local effects after implantation: Annex C, Test method for implantation in muscle' and in accordance with OECD principles of GLP.The implantation procedure was carried out under clean and aseptic conditions. Rabbits were anaesthetized using Ketamine (80mg/kg body weight)+Xylazine (5mg/kg body weight).the skin of anaesthetized rabbits were lightly swabbed with 70% alcohol and air dried. Five incisions were made on the skin and inserted five test implant materials (DTLAAS046-029) intramuscularly (para vertebral muscle) along one side of the spine (right side),and about 25 mm apart from each other. Similarly five control implant material (DTLAAS04-100C) was intramuscularly implanted in the contra lateral muscle (Left side) of each rabbits. The incision was then closed using sterile sutures. The study was conducted in 6 rabbits(whose para vertebral muscle was sufficiently large),3 animals each for 1& 4 weeks. The body weight of the animals was not less than 2kg. At the end of each observation period, animals were sacrificed and collected the implants (both control & test) with surrounding tissues for histopathological analysis. Macroscopically there was no hemorrhage, encapsulation, discoloration, necrosis or infection at the implant sites at any of the observation period. The general physical conditions of the experimental animals were normal. The increase in body weight and feed intake were normal and none of the animals showed any abnormality or behavioral changes during the experimental period.The histopathological comprehensive report is enclosed (TRPAT 022.Y13).The test material is found to be non irritant at one week and four weeks post implantation.Hence, the test material, polymer, DTLAAS046-029(WITOX D71.Y13) meet the requirements of the test as per "ISO 10993-6:2007(E): Biological evaluation of Medical devices: Part 6: Test for local effects after implantation: Annex C, Test method for implantation in muscle.

Blood Compatibility: The material was tested for blood compatibility. The tests conducted include hemolysis, complement activation and plasma coagulation. The details are provided in TRTRU132.Y13, provided in the annexures.

Hemolysis: The test samples were exposed to human blood collected from volunteers for 30 minutes under agitation at 35°C ± 2°C. The free hemoglobin liberated due to this contact between the samples and blood was estimated using spectro-photometric methods. The percentage hemolysis is estimated against the total hemoglobin present in the blood sample.The results are provided in table 7 below. Hemolysis upto 0.1% is normally observed due to experimental variations like blood sampling etc. Based on this it can be presumed that the material DTLAAS046 is non-hemolytic.

Test	Quantity (Mean ± SD)	Unit
Plasma Hb	5.4 ± 1.2	mg/dL
% Hemolysis	0.05 ± 0.01	%

Table 7: Blood damage (hemolysis) results

Complement Activation & Coagulation: The complement activation was assessed with commercially available ELISA kit by assessing the concentration of C3a factor in blood The coagulation modulation tendency was assessed by estimating the partial thromboplastin time (PTT) using a coagulation analyzer. The results are provided in table 8. There were no evidence of the material consistently affecting the coagulation pathways or complement activation.

Test	Quantity (Mean ± SD)	Unit
Complement Activation (C3a)	125% ± 190%	% change*
Partial Thromboplastin time	11.3%± 10%	% change

* - One sample showed substantial variation

Table 7: Complement activation and coagulation results

Sensitization: This study was designed to determine the skin sensitization potential in Guinea pigs by Maximization test (GPMT) of the physiological saline extract of polymer, DTLAAS046-054, intended to be used as implant used for rotator cuff repair for shoulder reconstruction.

The study was conducted in accordance with 'ISO 10993-10: 2010(E): Biological evaluation of medical devices-Part 10: Test for irritation and skin sensitization. Clause 7.5: Guinea pig Maximization test (GPMT) and in compliance with OECD principles of GLP. In this study there were 15 Guinea pigs for the extract (10 for test and 5 for control). The body weight range of the animals was 300-500g. The Physiological saline (PS) extract of test material (DTLAAS046-054) and control (PS alone) was intradermally injected and after seven days it was topically applied. Challenge test was carried out after fourteen days on all the animals. The appearance of the challenge skin sites of test and control animals were observed at 24, 48 and 72h after removal of dressings and patches. The skin reactions for erythrema and oedema were scored and recorded the numerical grading as per ISO 10993-10:2010(E).The result of the study indicated that the Physiological saline extract of the test material and control treated animals did not show any adverse skin reaction during the induction or challenge period and confirmed that the Physiological saline extract of the test material is non irritant at the laboratory conditions simulated. Hence, the Physiological saline extract of the test material, Polymer, DTLAAS046-054 (WITOX D72.Y13) meet the requirements of the test as per 'ISO 10993-10: 2010(E), Biological evaluation of medical devices- Part 10: Tests for irritation and skin sensitization: Clause 7.5: Guinea pig Maximization test(GPMT).

The results of the biological evaluation studies are summarized in table 8.

Serial No	Test	Detailed Report	Result
1	In-vitro cytoxicity - Direct Contact	TR TIC 032.Y13	Passed
2	In-vitro cytoxicity - Test on extract	TR MIC 031.Y13	Passed
3	Acute Systemic Toxicity- Saline Extract	TRTOX030.Y13	Passed
4	Acute Systemic Toxicity-Cotton Seed Oil Extract	TRTOX031.Y13	Passed
5	Animal Intracutaneous Reactivity	TRTOX034.Y13	Passed
6	Implantation in muscle	TRTOX053.Y13	Passed

7	Sensitization	TRTOX035.Y13	Passed
8	Hemocompatibility	TRTRU132.Y13	Passed

Table 8: Summary of results from Biological evaluation studies

5. Conclusions:

The biological, structural and stability studies on the fabric material for the rotator cuff repair device have been completed. The results are as summarized above and as detailed in the individual reports attached. The significance of the results are discussed in individual test reports.

Muraleedharan CV

Engineer G
Devices Testing Laboratory

Annexure:

1 Study Plan DTL AAS046.000
2 Test reports on Burst Strength - TR-AAS046.002, TR-AAS046.006
3 Test reports on Tensile Strength and Elongation at break - TR-AAS046.003, TR-AAS046.007
4 Test reports on Suture Retention Strength- TR-AAS046.004
5 Test reports on Water Permeability- TR-AAS046.005
6 Test reports on Material Identification(FTIR)- DPL/FTIR/16/13
7 Test reports on Trace Element Analysis- TRICP 002.Y13
8 Test reports on Thermogravimetry- TRLPA/TGA343.Y13, TRLPA/TGA360.Y13
9 Test reports on Differential Thermal Analysis(DTA)- TRLPA/DTA148.Y13, TRLPA/DTA154.Y13
10 Test reports on In-vitro cytoxicity- TR TIC 031.Y13, TR TIC 032.Y13
11 Test reports on Acute Systemic Toxicity- TRTOX030.Y13, TRTOX031.Y13
12 Test reports on Intracutaneous Reactivity- TRTOX034.Y13
13 Test reports on Implantation in Muscle TRTOX053.Y13

14 Test reports on Blood Compatibility- TR.TRU.132.Y13

15 Test reports on Sensitization- TRTOX035.Y13

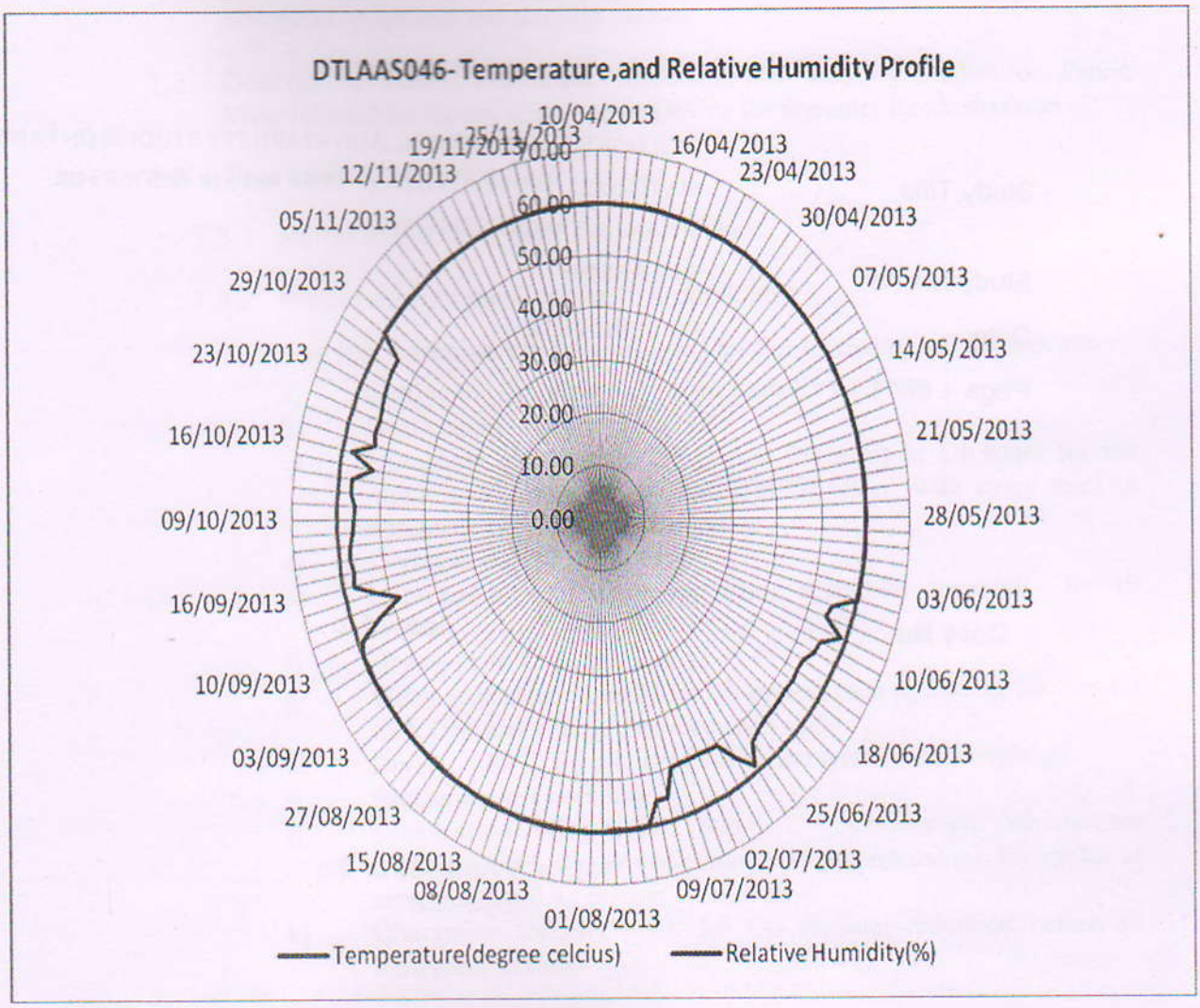

Figure 1: Temperature & Relative Humidity profile during the ageing period

BIOMEDICAL TECHNOLOGY WING

SREE CHITRA TIRUNAL INSTITUTE FOR MEDICAL SCIENCES AND TECHNOLOGY
Thiruvananthapuram, INDIA - 695 012

STUDY PLAN

Study Title	BIOLOGICAL, STRUCTURAL AND STABILITY STUDIES ON FABRIC MATERIAL USED FOR ROTARY CUFF REPAIR DEVICE FOR SHOULDER RECONSTRUCTION
Study number	DTL AAS 046
Date	08 June 2012

Copy No

Issued to

Name & Address of the Sponsor

The South India Textile Research Association, Coimbatore

1 Identification of study

1.1 Purpose: Characterization of the fabric material used for rotary cuff repair by biological, structural and stability studies

1.2 Descriptive Title : Biological, Structural and Stability Studies on Fabric Material used for Rotary Cuff Repair Device for Shoulder Reconstruction

1.3 Test Item & reference Item

1.3.1 Name of the Test Item: Polyester Fabric

1.3.2 Preparation of the Test Item, if applicable

The test samples will be prepared by the following set of operation

a) Cutting the samples to required sizes for each test
b) Heat setting to shape the fabric
c) Ultrasonic cleaning in 1% Extran (Neutral) in DI water for ten minutes, followed by 10 rinses in DI water, with every third in ultrasonic cleaner for five minutes
d) Drying at 60°C for 3 hours
e) Isopropyl alcohol extraction using a soxhlet apparatus for 10 cycles
f) Drying at 60°C for 6 hours
g) Water extraction at 60°C for 18 cycles, each cycles for 20 minutes with DI water change between each cycle
h) Drying at 60°C for 6 hours (Validation with repeat weighing)
i) Packaging in Tyvek bags
j) Sterilization using ETO, 600mg/L concentration, 90 minutes exposure, 60% RH in environment (noncondensing), six cycles of aeration with 0.22 micron filtered air
k) Quarantine for one week for EO residual reduction before all biological studies

1.3.3 Source of the Test Item:

SITRA, Coimbatore

1.3.4 Characterization of Test Item:

1.3.5 Name of the Reference Item:

Nil

1.3.6 Preparation of the Reference Item:

Nil

1.3.7 Source of the Reference Item:

Nil

2 Study Sponsor & Test Facility

2.1 Name and address of the sponsor.

Sri K Balasubramanian
Assistant Director & Head of Knitting and Weaving Division
The South India Textile Research Association, Coimbatore

2.2 Name of the study monitor:

Sri K Balasubramanian,

Assistant Director & Head of Knitting and Weaving Division

The South India Textile Research Association, Coimbatore

2.3 Name and address of the test facility and test sites involved:

Sree Chitra Tirunal Institute for Medical Sciences and Technology,
Biomedical Technology Wing, Poojappura,
Thiruvananthapuram- 695012, INDIA
Laboratory: Devices Testing Laboratory

2.4 Name and address of the study director

Sri. C.V Muraleedharan
Engineer G, Division of Artificial Organs

2.5 Name and address of the principal investigator, if any.

Sri. Ranjith G
Engineer C, Division of Artificial Organs

3 Proposed Study dates

3.1.1 Study Initiation date: June 20,2012

3.1.2 Dates of critical phases

3.1.3 Study completion date: April 20,2013

4 Experimental Protocol

4.1.1 Objectives:

Characterization of the fabric material used for rotary cuff repair by biological, structural and stability studies

4.1.2 Test Devices:

Polyester fabric

4.1.3 Study Design:

Mechanical Tests

The tests will be done as per the following standards

1. ISO 13934-1:Textiles-Tensile Properties of Fabrics
2. ISO 7198: Cardiovascular device: tubular grafts

Physico Chemical Tests

Material characterization is the crucial first step in the biological evaluation process. The extend of chemical characterization depends on what pre-clinical and clinical safety and toxicological data exist, and on the nature and duration of body contact with the medical device, but as a minimum the characterization shall address the constituent chemicals of the device and constituent residual process aids or additives used in its manufacture. The following physico-chemical tests would be done

1. Material Identification- By IR Spectroscopy
2. Trace Element Analysis

3. Thermogravimetry
4. Differential Thermal Analysis

Stability Studies

Accelerated ageing is the storing of packages at elevated temperature and / or other intensified environmental conditions in order to simulate real time ageing in a smaller duration of time. The increased temperature contributes towards a faster kinetics of package degradation. It is a proven fact that the ageing of medical device packaging can be accelerated to a two times pace by increasing the storage temperature by 10°C, by what is known as Q10 analysis. (A Q10 analysis involves testing of the package integrity at various temperatures and defining the difference in degradation rate for a 10°C rise in temperature). Based on the Q10 analysis on various types of packaging materials, it is now common practice to use a conservative value for Q10 = 2, for assessing the necessary period of accelerated ageing test for qualifying the package for a specific shelf life period.

Accelerated ageing factor is

$$AAF = Q_{10}^{(Te - Ta)/10}$$

where, Ta = Ambient storage température

Te = Accelerated ageing test temperature

Q_{10} = 2 (every 10°C rise doubles package degradation rate).

For the test device under consideration, following ageing conditions are arrived at

(a) Storage temperature: 30 ±2 °C

(b) Duration of simulated ageing: Five years

(c) Mean ageing temperature: 60°C (The ageing temperature is chosen to ensure that the material does not undergo any chemical

transformations during the ageing process and shall not exceed 65°C for polymeric materials)

AAF $= 2^{(60 - 30) / 10} = 8$

Based on the ageing factor, the ageing duration is estimated as (5*360)/8 =225` days.

(1) Accelerated ageing temperature: 60 °C ± 3 °C

(2) Relative humidity of the chamber: 60 % ± 20% RH

(3) Ageing duration: 225 days.

The following tests will be done after ageing

1. Thermogravimetry
2. Differential Thermal Analysis
3. Tensile Strength and elongation at break
4. Burst Strength

Biological Characterization

Biological Characterization will be done as per ISO 10993 standard. The ISO 10993 set entails a series of standards for evaluating the biocompatibility of a medical device prior to a clinical study. The following biological tests would be done

1. In Vitro Cytotoxicity
   - Test on extract
   - Direct contact
2. Acute Systemic Toxicity
3. Intracutaneous Reactivity
4. Intramuscular Implantation
5. Blood Compatibility
   - Hemolysis

- Platelet Adhesion and Activation
- Coagulation Profile
- Response to whole blood
- Complement Activation

6. Sensitization

5 Description of Methods

5.1.1 Type and frequency of tests, analyses and measurements to be made :

Mechanical Tests

Srl No	Name of Test	Standard followed	Number of Samples / test
1	Burst Strength	ISO 7198	Five
2	Tensile Strength and Elongation at break	ISO 13934-1	Five
3	Suture Retention	ISO 7198	Five
4	Permeability	ISO7198	Five

Physico Chemical

Srl No	Name of Test	Standard followed	Number of Samples / test
1	Material Identification	ASTM E1252	One
2	Trace Element Analysis(Heavy Metal Identification)	ICP-AES	One
3	Thermogravimetry	ASTM E1131	One
4	Differential Thermal Analysis	ASTM E537	One

Stability Studies (Tests done after Ageing)

Srl No	Name of Test	Standard followed	Number of Samples / test
1	Thermogravimetry	ASTM E1131	One
2	Differential Thermal Analysis	ASTM E537	One
3	Burst Strength	ISO7198	Five
4	Tensile Strength and Elongation at break	ASTM D5034	Five

Biological Characterization

Srl No	Name of Test	Standard followed	Number of Samples / test
1	In Vitro Cytotoxicity	ISO 10993-5:2009	1 set
2	Acute Systemic Toxicity	ISO 10993-11:2006	1 set
3	Intracutaneous Reactivity	ISO 10993-10:2010	1 set
4	Intramuscular Implantation	ISO 10993-6:2007	1 set
5	Blood Compatibility	ISO-10993-4:2002/AMD 1:2006	1 set
6	Sensitization	ISO 10993-10:2010	1 set

5.1.2 Proposed Study period

Sl. No	Phase of Study	Time period required
1	Accelerated ageing	225 days (7.5 months)
2	Completion of stability tests	1.5 months
3	Reporting	0.5 months
4	Total Study duration(including reporting)	9.5 months

5.1.3 Records maintained

5.1.3.1 Record of temperature and humidity every 3 hours during the entire time period of accelerated ageing

5.1.3.2 Observation sheet for recording of raw data

5.1.3.3 Test reports for individual tests

5.1.3.4 Master Schedule of Activities

6 References

6.1.1 ISO 10993-1:2009 Biological Evaluation of medical devise Part 1: Evaluation and testing in the risk management process

6.1.2 ISO 10993-4:2002/Amd 1:2006 Biological evaluation of medical devices Part 4: Selection of tests for interactions with blood

6.1.3 ISO 10993-5:2009 Biological evaluation of medical devices Part 5: Tests for in vitro cytotoxicity

6.1.4 ISO 10993-6:2007 Biological evaluation of medical devices Part 6: Tests for local effects after implantation

6.1.5 ISO 10993-8:2001 Biological evaluation of medical devices Part 8: Selection of reference materials

6.1.6 ISO 10993-10:2010 Biological evaluation of medical devices Part 10: Tests for irritation and delayed-type hypersensitivity

6.1.7 ISO 10993-11:2006 Biological evaluation of medical devices Part 11: Tests for systemic toxicity

6.1.8 ISO 10993-13:1998 Biological evaluation of medical devices Part 13: Identification and quantification of degradation products from polymeric medical devices

6.1.9 ISO/TS 10993-19:2006 Biological evaluation of medical devices Part 19: Physico-chemical, morphological and topographical characterization of materials

6.1.10 ASTM E1252-98(2007) Standard Practice for General Techniques for Obtaining Infrared Spectra for Qualitative Analysis

6.1.11 E1131-08 Standard Test Method for Compositional Analysis by Thermogravimetry

6.1.12 ASTM E537-98 Standard Test Method for Assessing the Thermal Stability of Chemicals By Methods of Thermal Analysis

6.1.13 Charles J Petit, Robert Boswell, Andrew Mahar,James Tasto and Robert A Pedowitz, Biomechanical Evaluation of a new technique for Rotary Cuff Repair, The Amer.J.Sports Med.,Vol 31,No.6

6.1.14 The European Agency for the Evaluation of Medicinal Products. Guideline for stability testing : stability testing of existing active substances and related finished products, CPMP/QWP/122/02 rev 01 , Dec 2003

7 Confidentiality

Sree Chitra Tirunal Institute for Medical Sciences and Technology undertakes not to divulge or disclose to any third party the nature or results of this study without the written prior consent of the Sponsor.

8 Archiving materials & records

No samples will be archived by the Institute at the end of the study. If the samples are to be archived, this shall be communicated to the Institute and the additional expenses for archiving the samples bourn by the Sponsor. All unused samples will be returned to the sponsor at the end of the study.

9 Format of final report

The report shall contain at least the following details.

Title:

Report No and Date:

Summary of the Study carried out:

Protocol used:

Observations and results:

Report of individual tests:

Conclusion (s):

Name & signature of the Study Director:

Date of Report:

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TEST REPORT

Date : 24/04/13 Report No : TR AAS 046.002 Total Number of Pages : 3

1.	Name of the Laboratory :	DEVICE TESTING LABORATORY Division of Artificial Organs
2.	Work Order Number :	DTL AAS046
3.	Customer Sample Code :	Nil
4.	Date of receipt of sample :	10-05-2012
5.	Date / Period of conduct of test :	24/04/13
6.	Test method used :	ISO7198
7.	Description of the sample :	Polyester Fabric for Rotator Cuff Repair Device
8.	Details of specimen preparation :	Sample prepared as described in main report(No Ageing)
9.	Result (with units of measurement) :	(see report for details)

Declaration

I here by certify that this test certificate is for the sample received as per the above work order number.

10.	Name and Signature of the Scientist in Charge :	Muraleedharan CV Engineer G

Report on

Assessment of Burst Strength of Polyester Fabric

Work Order Reference : DTL AAS046

1 SCOPE

This study covers an assessment of the burst strength of the fabric material by mechanical testing based on ISO 7198: Cardiovascular Implants: Tubular Vascular Prosthesis Section 8.3.3 Determination of Burst Strength .

2. TEST CONDITIONS

The study was conducted at room temperature.

3 PROCEDURE

The test was conducted on 6 samples with dimensions 2.5 cm X 2.5 cm. An area of the sample is clamped over an orifice by means of a flat annular clamp ring and a cylindrical probe with a hemispherical head is traversed through the specimen until it ruptures. The test measures the force required for rupture. A special fixture used for testing the burst strength of vascular grafts was used for the purpose. The fixture consists of a base where the sample can be stretched and held rigid. A metallic cylindrical probe of diameter 7.8mm with a hemispherical end is used to pierce the fabric. The above fixture was placed on an UTM and a compressive force was applied at a constant rate of compression 125mm/min. The peak compressive load at which burst occurs is taken as the burst load. The burst strength is calculated by dividing the burst load by the area of the probe in contact with the fabric(area of hemisphere with diameter 7.8 mm). The details of the equipment are as below

- Name of Equipment: Instron UTM model 3345
- Load Cell: 5 kN- Model No 66515
- Software: Blue Hill Version2.22.773
- Compression Platen- S5636A
- Probe diameter- 7.8mm
- Probe area-95.6 mm^2

4 TEST SAMPLES

Sl. No	Sample Id
1	DTL AAS046.002

5. OBSERVATIONS

The test results are as listed below.

Sl. No.	Maximum load (N)	Burst Strength (N/mm^2)
1	218.05	2.28
2	209.07	2.19
3	252.37	2.64
4	164.15	1.72
5	181.73	1.90
6	146.05	1.53
Mean		2.04
Standard Deviation		0.41

7. CONCLUSION

The burst strength has been conducted on the samples and the results are as indicated above.

Test Done by		Verified and reported by	
Name	Signature	Name	Signature
Arunlal C L		Ranjith G	

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TEST REPORT

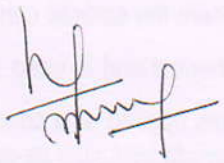
Date : 5/12/13 Report No : TR AAS 046.006 Total Number of Pages : 3

1.	Name of the Laboratory :	DEVICE TESTING LABORATORY Division of Artificial Organs
2.	Work Order Number :	DTL AAS046
3.	Customer Sample Code :	Nil
4.	Date of receipt of sample :	10-05-2012
5.	Date / Period of conduct of test :	5/12/13
6.	Test method used :	ISO7198
7.	Description of the sample :	Polyester Fabric for Rotator Cuff Repair Device
8.	Details of specimen preparation :	Sample prepared as described in main report and undergone accelerated ageing
9.	Result (with units of measurement) :	(see report for details)

Declaration

I here by certify that this test certificate is for the sample received as per the above work order number.

10.	Name and Signature of the Scientist in Charge :	Muraleedharan CV Engineer G



Report on

Assessment of Burst Strength of
Polyester Fabric

Work Order Reference : DTL AAS046

1. SCOPE

This study covers an assessment of the burst strength of the fabric material by mechanical testing based on ISO 7198: Cardiovascular Implants: Tubular Vascular Prosthesis Section 8.3.3 Determination of Burst Strength

2. TEST CONDITIONS

The study was conducted at room temperature.

3 PROCEDURE

The test was conducted on 6 samples with dimensions 2.5 cm X 2.5 cm. An area of the sample is clamped over an orifice by means of a flat annular clamp ring and a cylindrical probe with a hemispherical head is traversed through the specimen until it ruptures. The test measures the force required for rupture. A special fixture used for testing the burst strength of vascular grafts was used for the purpose. The fixture consists of a base where the sample can be stretched and held rigid. A metallic cylindrical probe of diameter 7.8mm with a hemispherical end is used to pierce the fabric. The above fixture was placed on an UTM and a compressive force was applied at a constant rate of compression 125mm/min. The peak compressive load at which burst occurs is taken as the burst load. The burst strength is calculated by dividing the burst load by the area of the probe in contact with the fabric(area of hemisphere with diameter 7.8 mm). The details of the equipment are as below

- Name of Equipment: Instron UTM model 3345
- Load Cell: 5 kN- Model No 66515
- Software: Blue Hill Version2.22.773
- Compression Platen- S5636A
- Probe diameter- 7.8mm
- Probe area-95.6 mm^2

4 TEST SAMPLES

Sl. No	Sample Id
1	DTL AAS046.076

5. OBSERVATIONS

The test results are as listed below.

Sl. No.	Maximum load (N)	Burst Strength (N/mm^2)
1	222.89	2.33
2	201.93	2.11
3	187.59	1.96
4	199.35	2.09
5	273.42	2.86
6	204.02	2.13
Mean		2.25
Standard Deviation		0.32

7. CONCLUSION

The burst strength has been conducted on the samples and the results are as indicated above.

Test Done by		Verified and reported by	
Name	Signature	Name	Signature
Arunlal C L		Ranjith G	

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TEST REPORT

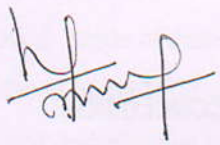
Date : 20/04/13 Report No : TR AAS 046.003 Total Number of Pages : 3

1.	Name of the Laboratory :	DEVICE TESTING LABORATORY Division of Artificial Organs
2.	Work Order Number :	DTL AAS046
3.	Customer Sample Code :	Nil
4.	Date of receipt of sample :	10-05-2012
5.	Date / Period of conduct of test :	20/04/13
6.	Test method used :	ISO13934-1
7.	Description of the sample :	Polyester Fabric for Rotator Cuff Repair Device
8.	Details of specimen preparation :	Sample prepared as described in main report(No Ageing)
9.	Result (with units of measurement) :	(see report for details)

Declaration

I here by certify that this test certificate is for the sample received as per the above work order number.

10.	Name and Signature of the Scientist in Charge :	Muraleedharan CV Engineer G



Report on

Assessment of Tensile Strength and Elongation at Break of Polyester Fabric

Work Order Reference : DTL AAS046

1 SCOPE

This study covers an assessment of the tensile strength and elongation at break of the fabric material by mechanical testing based on ISO 13934-1:Textiles-Tensile Properties of Fabrics

2. TEST CONDITIONS

The study was conducted at room temperature.

3 PROCEDURE

The test was conducted on 5 samples with dimensions 10cm x 5 cm. The fabric material was stretched at a constant rate of extension of 50mm/min. The tensile extension was applied using a UTM. The gauge length is fixed as 5cm. The details of the equipment are as below

- Name of Equipment: Instron UTM model 3345
- Load Cell: 5 kN- Model No 66515
- Software: Blue Hill Version2.22.773
- Test Grip: 5KN- Model No.2710-105

4 TEST SAMPLES

Sl. No	Sample Id
1	DTL AAS046.001

5. OBSERVATIONS

The test results are as listed below. The force corresponding to the peak of the load extension graph is taken as the maximum force. The point at which the force drops by 2% of the maximum force has been chosen as the break point. The elongation at this point is reported as the elongation at break. The % elongation is calculated by dividing the elongation by the gauge length and expressing in percent.

Srl No	Maximum Force (Newton)	Elongation at break (%)
1	571.21	81.98
2	702.48	111.68
3	654.51	107.32
4	678.51	84.46
5	672.73	69.48

7. CONCLUSION

The tensile test has been conducted on the samples and the results are as indicated above.

Test Done by		Verified and reported by	
Name	Signature	Name	Signature
Arunlal C L		Ranjith G	

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TEST REPORT

Date : 4/12/13 Report No : TR AAS 046.007 Total Number of Pages : 3

1.	Name of the Laboratory :	DEVICE TESTING LABORATORY Division of Artificial Organs
2.	Work Order Number :	DTL AAS046
3.	Customer Sample Code :	Nil
4.	Date of receipt of sample :	10-05-2012
5.	Date / Period of conduct of test :	4/12/13
6.	Test method used :	ISO13934-1
7.	Description of the sample :	Polyester Fabric for Rotator Cuff Repair Device
8.	Details of specimen preparation :	Sample prepared as described in main report and undergone accelerated ageing
9.	Result (with units of measurement) :	(see report for details)

Declaration

I here by certify that this test certificate is for the sample received as per the above work order number.

10.	Name and Signature of the Scientist in Charge :	Muraleedharan CV Engineer G

Report on

Assessment of Tensile Strength and Elongation at Break of Polyster Fabric

Work Order Reference : DTL AAS046

1 SCOPE

This study covers an assessment of the tensile strength and elongation at break of the fabric material by mechanical testing based on ISO 13934-1:Textiles-Tensile Properties of Fabrics

2. TEST CONDITIONS

The study was conducted at room temperature.

3 PROCEDURE

The test was conducted on 5 samples with dimensions 10cm x 5 cm which had undergone accelerated ageing. The fabric material was stretched at a constant rate of extension of 50mm/min. The tensile extension was applied using a UTM. The gauge length was fixed at 5cm. The details of the equipment are as below

- Name of Equipment: Instron UTM model 3345
- Load Cell: 5 kN- Model No 66515
- Software: Blue Hill Version2.22.773
- Test Grip: 5KN- Model No.2710-105

4 TEST SAMPLES

Sl. No	Sample Id
1	DTL AAS046.077

5. OBSERVATIONS

The test results are as listed below. The force corresponding to the peak of the load extension graph is taken as the maximum force. The point at which the force drops by 2% of the maximum force has been chosen as the break point. The elongation at this point is taken as the elongation at break. The % elongation is calculated by dividing the elongation at break by the gauge length(5 cm)

Srl No	Maximum Force (Newton)	Elongation at break (%)
1	671.62	68.98
2	432.19	115.00
3	553.42	71.16
4	394.45	109.34
5	533.33	86.34

7. CONCLUSION

The tensile test has been conducted on the aged samples and the results are as indicated above.

Test Done by		Verified and reported by	
Name	Signature	Name	Signature
Arunlal C L		Ranjith G	

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TEST REPORT

Date : 29/04/13 Report No : TR AAS 046.004 Total Number of Pages : 3

1.	Name of the Laboratory :	DEVICE TESTING LABORATORY Division of Artificial Organs
2.	Work Order Number :	DTL AAS046
3.	Customer Sample Code :	Nil
4.	Date of receipt of sample :	10-05-2012
5.	Date / Period of conduct of test :	29/04/13
6.	Test method used :	ISO7198
7.	Description of the sample :	Polyester Fabric for Rotator Cuff Repair Device
8.	Details of specimen preparation :	Sample prepared as described in main report(no ageing)
9.	Result (with units of measurement) :	(see report for details)

Declaration

I here by certify that this test certificate is for the sample received as per the above work order number.

10.	Name and Signature of the Scientist in Charge :	Muraleedharan CV Engineer G

Report on

Assessment of Suture Retention Strength of Polyester Fabric

Work Order Reference : DTL AAS046

1 SCOPE

This study covers an assessment of the suture retention strength of the fabric material by mechanical testing based on ISO 7198: Cardiovascular Implants: Tubular Vascular Prosthesis Section 5.8 Suture Retention Strength

2. TEST CONDITIONS

The study was conducted at room temperature.

3 PROCEDURE

The test was conducted on 5 samples with dimensions 2.5 cm X 2.5 cm. The test measures the force necessary to pull a suture from the fabric or cause the wall of the fabric to fail under tensile extension. One end of the sample is clamped to the lower jaw of the UTM and at the other end a suture is inserted 2mm from the edge. The ends of the suture are clamped to the upper jaw of the UTM. A tensile extension at the rate of 50mm/min is applied on the UTM. Three standard sizes of sutures were used(USP 0,USP 2-0 and USP 3-0) on every sample. The details of the equipment are as below

- Universal Testing Machine Model 3345 (M/S Instron Corporation, USA)
- Load Cell: 5KN- Model No. 66515 (M/S Instron Corporation, USA)
- Software : Blue Hill Version V2.22.773 (M/S Instron Corporation, USA)
- Test Grip: 5KN- Model No.2710-105 and 2714-004 (M/S Instron Corporation, USA)
- Extension Rate 50 mm/min
- Suture Sizes used- USP 0(Silk sutures),USP 2-0(Polyester Sutures),USP 3-0(Polyester sutures)

Archives

All the study related raw data together with the copy of final report was archived in the GLP archives of SCTIMST for five years. After the completion of this period sponsor consent will be sought to either extend the archive periods or return the archived material to the sponsor for the disposal of the material.

Report prepared by	:	Ms. Geetha CS
Test done and Report checked by	:	Dr. Gayathri V
Verified and Reported by	:	Dr. PV Mohanan

Table 1: Common clinical signs and observation sheet – Cotton Seed Oil extract.
Expt. No: EXTOX/AS/013.Y13 **Date:** 09.04.13 to 12.04.13

Clinical observation*	Animals	Test					Control				
		1♂	2♂	3♂	4♂	5♂	6♀	7♀	8♀	9♀	10♀
Body weight(g)	Initial	23.00	20.60	20.85	22.25	21.45	19.65	21.45	21.55	21.55	23.00
Vol. of Injection	mL	1.2	1.0	1.0	1.1	1.1	1.0	1.1	1.1	1.1	1.2
Respiratory	Imm./4h	N	N	N	N	N	N	N	N	N	N
	24h	N	N	N	N	N	N	N	N	N	N
	48h	N	N	N	N	N	N	N	N	N	N
	72h	N	N	N	N	N	N	N	N	N	N
Motor	Imm./4h	N	N	N	N	N	N	N	N	N	N
	24h	N	N	N	N	N	N	N	N	N	N
	48h	N	N	N	N	N	N	N	N	N	N
	72h	N	N	N	N	N	N	N	N	N	N
Convulsion	Imm./4h	N	N	N	N	N	N	N	N	N	N
	24h	N	N	N	N	N	N	N	N	N	N
	48h	N	N	N	N	N	N	N	N	N	N
	72h	N	N	N	N	N	N	N	N	N	N
Reflexes	Imm./4h	N	N	N	N	N	N	N	N	N	N
	24h	N	N	N	N	N	N	N	N	N	N
	48h	N	N	N	N	N	N	N	N	N	N
	72h	N	N	N	N	N	N	N	N	N	N
Ocular signs	Imm./4h	N	N	N	N	N	N	N	N	N	N
	24h	N	N	N	N	N	N	N	N	N	N
	48h	N	N	N	N	N	N	N	N	N	N
	72h	N	N	N	N	N	N	N	N	N	N
Cardiovascular signs	Imm./4h	N	N	N	N	N	N	N	N	N	N
	24h	N	N	N	N	N	N	N	N	N	N
	48h	N	N	N	N	N	N	N	N	N	N
	72h	N	N	N	N	N	N	N	N	N	N
Salivation	Imm./4h	N	N	N	N	N	N	N	N	N	N
	24h	N	N	N	N	N	N	N	N	N	N
	48h	N	N	N	N	N	N	N	N	N	N
	72h	N	N	N	N	N	N	N	N	N	N

Piloerection	Imm./4h	N	N	N	N	N	N	N	N	N	N
	24h	N	N	N	N	N	N	N	N	N	N
	48h	N	N	N	N	N	N	N	N	N	N
	72h	N	N	N	N	N	N	N	N	N	N
Analgesia	Imm./4h	N	N	N	N	N	N	N	N	N	N
	24h	N	N	N	N	N	N	N	N	N	N
	48h	N	N	N	N	N	N	N	N	N	N
	72h	N	N	N	N	N	N	N	N	N	N
Muscle tone	Imm./4h	N	N	N	N	N	N	N	N	N	N
	24h	N	N	N	N	N	N	N	N	N	N
	48h	N	N	N	N	N	N	N	N	N	N
	72h	N	N	N	N	N	N	N	N	N	N
Gastrointestinal	Imm./4h	N	N	N	N	N	N	N	N	N	N
	24h	N	N	N	N	N	N	N	N	N	N
	48h	N	N	N	N	N	N	N	N	N	N
	72h	N	N	N	N	N	N	N	N	N	N
Skin	Imm./4h	N	N	N	N	N	N	N	N	N	N
	24h	N	N	N	N	N	N	N	N	N	N
	48h	N	N	N	N	N	N	N	N	N	N
	72h	N	N	N	N	N	N	N	N	N	N
Body weight(g)	Final	29.35	24.95	25.90	28.50	29.05	24.45	25.90	25.75	25.90	27.35

* As per ISO 10993-11: 2006 (E) .

Table 2: Mortality details

No	Group	Dose (mL/kg)	Death					Total no of death
			Immediate	4 h	24 h	48 h	78 h	
1	Control	50	NIL					
2	Test extract	50	NIL					

Table 3: Common clinical signs and observations

Clinical observation	Observed sign	Involved system (s)
Respiratory	Dyspnea (abdominal breathing, gasping), aponoea, cyanosis, tachypenea, nostril discharges	CNS, pulmonary, cardiac
Motor	Decrease/increase somnolence, loss of righting, anesthesia, catalepsy ,ataxia, unusual locomotion, prostration, tremors, fasciculation	CNS, somatomotor, sensory, neuromuscular, automatic respiratory
Convulsion	Clonic, tonic, tonic-clonic, asphyxial opisthotonos	CNS, neuromuscular, autonomic, respiratory
Reflexes	Corneal, righting, myotact, light, startle reflex	CNS, sensory, autonomic, neuromuscular
Ocular signs	Lacrimation, miosis, mydriasis, exophthalmos, ptosis, opacity, iritis, conjunctivitis, chromodacryorrhea, relaxatrion of nicititating membrane	Autonomic, irritation
Cardiovsacular signs	Bradycardia, tachycardia, arrhythmia, vasodialation, vasoconstriction	CNS, autonomic, cardiac, pulmonary
Salivation	Excessive	Autonomic
Piloerection	Rough hair	Autonomic
Analgesia	Decrease reaction	CNS,sensory
Muscle tone	Hypotonia, hypertonia	Autonomic
Gastrointestinal	Soft stool, diarrhea, emesis, diuresis, rhinorrhea	CNS, autonomic, sensory, GI motility, Kidney
Skin	Edema, Erythema	Tissue damage and irritation

----------End of Test Report: TRTOX031.Y13----------

SREE CHITRA TIRUNAL INSTITUTE FOR MEDICAL SCIENCES AND TECHNOLOGY

(An Institute of National Importance under Govt. of India)

BIOMEDICAL TECHNOLOGY WING

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Phone: 91-471-234-0801, Fax: 91-471-234-1814, E-mail: csc@sctimst.ac.in

TEST REPORT (INTERNAL)

Name of Test / Study	:	Animal Intracutaneous (Intradermal) reactivity test of Physiological Saline and Cotton seed oil extracts of Polymer, DTLAAS046-025 in albino Rabbits.
Work order/Study number	:	WITOX D73.Y13
Test material code	:	DTLAAS046-025
Toxicology Sample ID	:	TSTOX 022.Y13
Date of receipt of sample	:	01.04.13
Period of conduct of test	:	24.04.2013 to 02.05.2013
Test Report Number	:	TRTOX 034.Y13
Date	:	08.05.2013

Test Facility

Toxicology Division
Biomedical Technology Wing,
Sree Chitra Tirunal Institute for Medical Sciences and Technology,
Thiruvananthapuram 695 012, Kerala, India

Name and Address of Internal Customer

Mr. CV Muraleedharan
Device Testing Lab

Name of the Study Director: Dr. PV. Mohanan

Declaration

I hereby certify that this test certificate is for the sample received as per the above work order number

Authorized Signatory : Dr. P. V. Mohanan, SIC, Toxicology Division.
(Name & Designation)

cofrac ESSAIS N° 1-1433

GLP COMPLIANCE STATEMENT

Study No.	:	WITOX D73.Y13
Test material code	:	DTLAAS046-025
Study Title	:	Animal Intracutaneous (Intradermal) reactivity test of Physiological saline and Cotton seed oil extracts of Polymer, DTLAAS046-025 in albino Rabbits.

All the data presented in this report are original, correct and accurate to the best of my knowledge. I hereby attest that this study was conducted in compliance with the OECD Principles of GLP.

The study was conducted to meet the requirements of the 'ISO 10993-10: 2010 (E), Biological evaluation of medical devices -- Part 10: Test for irritation and skin sensitization test, Clause 6.4: Animal Intracutaneous (Intradermal) reactivity test and USP34/ NF29: 2011.

Study Director

QUALITY ASSURANCE STATEMENT

This is to certify that to the best of signatories' knowledge, the above test report reflects the raw data as per the requirements of Good Laboratory Practices and Quality Assurance System as per ISO 17025 presently understood and practiced in the Biomedical Technology Wing, SCTIMST, Thiruvananthapuram.

Dr. P. Ramesh
Quality Manager

PERSONNEL INVOLVED IN THE STUDY

Study Director	:	Dr. P V. Mohanan
Study Personnel	:	Ms. Geetha CS
		Dr. V Gayathri
		Ms. Anju Mohan

SUMMARY

The study was designed to determine the irritation potential of the physiological saline and cotton seed oil extracts of the test material, Polymer, DTLAAS046-025 intended to be used for implant used for rotary cuff repair for shoulder reconstruction, following intradermal injection in albino Rabbits.

The study was conducted in accordance with 'ISO 10993-10: 2010 (E), Biological evaluation of medical devices – part 10: Test for irritation and skin sensitization, Clause 6.4: Animal Intracutaneous (Intradermal) reactivity test and USP 34/ NF 29:2011 and in compliance with OECD principles of GLP.

In this study there were 3 Rabbits for each material. Healthy adult animals not less than 2kg and not previously used were used for the test.

The physiological saline (PS) and cotton seed oil (CSO) extracts of the test material was aseptically injected into 5 sites (0.2mL/site) on the upper left hand side and right hand side of 3 Rabbits. The physiological saline (control) alone and cotton seed oil (control) alone were injected into 5 sites on the lower left hand side and lower right hand side of the same Rabbits. The grading of erythema and oedema of test and control sites of all animals at 24, 48 and 72h were recorded as per ISO 10993-10: 2010 (E).

The results (Table 1 & 2) indicated that the physiological saline and cotton seed oil extracts of the test material, DTLAAS046-025 (WITOX D73.Y13) produced a total mean score of '0' in physiological saline extract and '0.26' in Cotton seed oil extract following intradermal injection.

Hence, the test material, Polymer, DTLAAS046-025 meets the requirements of the test as per ISO 10993-10: 2010 (E) - Biological evaluation of medical devices: Part 10: Tests for irritation and skin sensitization: Clause 6.4: Animal Intracutaneous (Intradermal) reactivity test.

Introduction

The study, (WITOX D73.Y13) was designed for the Animal Intracutaneous (Intradermal) reactivity tests of physiological saline (PS) and cotton seed oil (CSO) extracts of Polymer, DTLAAS046-025 in albino Rabbits.

The study was conducted in accordance with ISO 10993-10:2010 (E) - Biological evaluation of medical devices - Part 10. Tests for irritation and skin sensitization: Clause 6.4: Animal Intracutaneous (Intradermal) reactivity test and USP 34/ NF 29:2011 and in compliance with OECD principles of GLP.

The grading of erythema and oedema of test and control sites of all animals at 24, 48 and 72h were recorded as per Table 3.

Identification of study

Title

Animal Intracutaneous (Intradermal) reactivity test of physiological saline and Cotton seed oil extracts of Polymer, DTLAAS046-025 in albino Rabbits.

Objective

To assess the potential of the Physiological saline and Cotton seed oil extracts of Polymer, DTLAAS046-025 to produce irritation following intradermal injection in Rabbits.

Test item details

The sponsor is responsible for the test substance purity, identity, stability and other required data. The details of the test substance provided by the sponsor are the following:

Name of test item	:	DTLAAS046-025
Preparation of test item	:	The material is cut from fabric, cleaned in ultrasonic cleaner, extracted in isopropyl alcohol and sterilized in ETO.
Nature of materials	:	Polymer
Physical Appearance	:	Fabric
Manufacturer/Source of test item /Supplied by	:	Device Testing Lab, BMT Wing, SCTIMST
Batch/Lot Number	:	NA
Trade Name	:	NA
Sterilization	:	ETO
Package	:	Packed
Storage conditions	:	Room Temperature
Handling	:	Aseptic conditions

Reference Item details

Name of reference item	:	1. Physiological Saline	2.Cotton seed oil
Preparation	:	Commercially available	Commercially available
Nature of materials	:	Liquid	Liquid
Physical Appearance	:	Clear solution	Clear viscous solution
Manufacturer/Source	:	M/s Parentral Drugs Ltd.India	Sigma-Aldrich
Supplied by		M/s Sreeja Medicals, TVM	Sigma-Aldrich
Batch/Lot Number	:	2C-292	MKBG0088V
Sterilization	:	Sterile	Dry heat
Package	:	Bottle	Bottle
Storage conditions	:	Room Temperature	Room Temperature
Handling	:	Aseptic conditions	Aseptic conditions

Name of the study monitor : Nil

Study dates

Study Initiation	:	01.04.2013
Initiation of experiment	:	24.04.2013
Completion of experiment	:	02.05.2013
Study completion	:	08.05.2013

Test Methods

ISO 10993-10: 2010 (E) - Biological Evaluation of medical devices -- Part 10: Tests for irritation and skin sensitization: Clause 6.4: Animal Intracutaneous (Intradermal) reactivity test and USP 31/ NF 26.

Experimental Protocol

Test system Details

Name of Species	:	Rabbit
Strain of the Animal	:	Albino
Sex	:	Male/ Female
Weight at start of treatment	:	Not less than 2kg
Age	:	Adult
No. of Animals per material	:	3
Source of supply	:	Division of Laboratory Animal Sciences, BMT Wing, SCTIMST
Room where test performed	:	Procedure room.

Justification of the Test System

ISO 10993-10: 2010 (E), Clause 6.4 recommends the usage of Rabbits for Intracutaneous (Intradermal) reactivity test by intradermal injection and also Rabbits are reported to be a suitable model for pre-clinical safety evaluation. Historically Rabbits have been shown to be sensitive to the toxicological potential of a variety of compounds including chemicals, pesticides and drugs. Handling and availability of this animal is very easy.

Test system Identification

Each animal was housed individually and identified with labels on each cage and tattoo marks on animals ears. Label has animal number, experiment number, group, experiment initiation and experiment completion date.

Quarantine procedures

Since the animals were from our colony, there will be no quarantine for these animals.

Acclimatization

The animals were acclimatized for a period of 5 days before initiation of experiment.

Animal Husbandry and welfare

All animals were handled humanely, without making pain or distressing and with due care for their welfare. Animals care and management complied with the regulations of the Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA), Govt. of India. Institutional Animal Ethics Committee's approval was taken before initiating animal experiments (Approval No: SCT/IAEC-040/DECEMBER/2012/79).

Equipments/instruments used

Disposable syringes (Batch No. 237014G31), Laminar bench (EQTOX019), pH meter (EQTOX074), Incubator shaker (EQTOX 628).

Experimental animal room sanitation

In addition to daily cleaning with disinfectant solution, the animal room was decontaminated before initiation of each experiment.

Environmental conditions

Animals were maintained in a controlled environment with a temperature 22 $\pm$ 3^{0}C, humidity of 30 - 70 % and a light/ dark cycle of 12 h. There were a minimum of 15 fresh air changes per hour.

Housing Conditions

The animals were housed individually in clean stainless steel fabricated cages.

Feed and water

The animals were provided with commercially available feed for Rabbits, and Aqua guard filtered fresh drinking water *ad libitum*. The feed and water were analyzed every six months.

Fasting

Post medication fasting was not required.

Justification of the method

As per regulatory requirements, the dose route has been chosen to characterize the toxicological profile of the test extract.

Test/ Reference Item Administration

Delivery system	:	Extract.
Method	:	Intradermal injection.
Preparation for administration	:	Physiological saline and Cotton seed oil extracts as per ISO 10993-12: 2012 (E)
Frequency of administration	:	0.2 mL/5 sites
Amount of extract required	:	20 mL

Experimental Procedure

Extraction of material

Test item	:	DTLAAS046-025	
		Test	Control
Surface area(cm^2)/weight of sample(g)	:	60 cm^2	-
Extraction temperature	:	$50^0C \pm 2^0C$	$50^0C \pm 2^0C$
Extraction media	:	1. Physiological saline 2. Cotton seed oil	1. Physiological saline 2. Cotton seed oil
Volume of media	:	1. 20+4 (absorption capacity) mL 2. 20+5 (absorption capacity) mL	1. 20mL 2. 20 mL
Period of extraction	:	72h ±2h	72 h ±2h
No of replicates	:	1	1
Speed of agitation	:	50 rpm	50 rpm
pH of the extract	:	1. 6.3 2. NA	1. 6.2 2. NA

Results and conclusion

The results (Table 1 & 2) indicated that the physiological saline and Cotton seed oil extracts of the test material, Polymer, DTLAAS046-025 in albino Rabbits (WITOX D73.Y13) produced a total mean score of '0' in physiological saline extract and '0.26' in cotton seed oil extract, following Intradermal injection.

Hence, the test material, Polymer, DTLAAS046-025 in albino Rabbits meet the requirements of the test as per ISO 10993-10: 2010 (E) - Biological evaluation of medical devices -- Part 10: Tests for irritation and skin sensitization: Animal Intracutaneous (Intradermal) reactivity test.

Study Plan Alteration (Amendment, Deviation): Nil

Good Laboratory Practices

The study was conducted in accordance to OECD principles of GLP and as per agreed protocol.

Archives

All the study related raw data together with the copy of final report was archived in the GLP archives of SCTIMST for five years. After the completion of this period sponsor consent will be sought to either extend the archive periods or return the archived material to the sponsor for the disposal of the material.

Test done by	:	Ms. Geetha CS
Report prepared by	:	Dr. V Gayathri
Report Checked by	:	Ms. Anju Mohan
Verified and Reported by	:	Dr. PV Mohanan

TABLE 1: Observation sheet

EXPT NO: EXTOX/IC/008.Y13 DATE: 29.04.13 to 02.05.13

Sl. No.	Animal ID.No		Observation at 24 h	Observation at 48 h	Observation at 72 h
1.	093♀	Erythema	Test site CSO=0,0,0,0,0 NS =0,0,0,0,0	CSO=0,1,1,1,1 NS = 0,0,0,0,0	CSO= 0,0,0,0,0 NS = 0,0,0,0,0
			Control site CSO= 0,0,0,0,0 NS = 0,0,0,0,0	CSO= 0,0,0,0,0 NS = 0,0,0,0,0	CSO= 0,0,0,0,0 NS = 0,0,0,0,0
		Oedema	Test site CSO= 0,0,0,0,0 NS =0,0,0,0,0	CSO= 0,0,0,0,0 NS = 0,0,0,0,0	CSO= 0,0,0,0,0 NS = 0,0,0,0,0
			Control site CSO= 0,0,0,0,0 NS = 0,0,0,0,0	CSO= 0,0,0,0,0 NS = 0,0,0,0,0	CSO= 0,0,0,0,0 NS = 0,0,0,0,0
2.	161 ♀	Erythema	Test site CSO= 0,0,0,0,0 NS = 0,0,0,0,0	CSO= 1,1,1,1,1 NS = 0,0,0,0,0	CSO= 0,0,0,0,0 NS = 0,0,0,0,0
			Control site CSO= 0,0,0,0,0 NS = 0,0,0,0,0	CSO= 0,0,0,0,0 NS = 0,0,0,0,0	CSO= 0,0,0,0,0 NS = 0,0,0,0,0
		Oedema	Test site CSO= 0,0,0,0,0 NS = 0,0,0,0,0	CSO= 0,0,0,0,0 NS = 0,0,0,0,0	CSO= 0,0,0,0,0 NS = 0,0,0,0,0
			Control site CSO= 0,0,0,0,0 NS = 0,0,0,0,0	CSO= 0,0,0,0,0 NS = 0,0,0,0,0	CSO= 0,0,0,0,0 NS = 0,0,0,0,0
3.	188♂	Erythema	Test site CSO= 0,0,0,0,0 NS = 0,0,0,0,0	CSO= 0,0,1,1,1 NS = 0,0,0,0,0	CSO= 0,0,0,0,0 NS = 0,0,0,0,0
			Control site CSO= 0,0,0,0,0 NS = 0,0,0,0,0	CSO= 0,0,0,0,0 NS = 0,0,0,0,0	CSO= 0,0,0,0,0 NS = 0,0,0,0,0
		Oedema	Test site CSO= 0,0,0,0,0 NS = 0,0,0,0,0	CSO= 0,0,0,0,0 NS = 0,0,0,0,0	CSO= 0,0,0,0,0 NS = 0,0,0,0,0
			Control site CSO= 0,0,0,0,0 NS = 0,0,0,0,0	CSO= 0,0,0,0,0 NS = 0,0,0,0,0	CSO= 0,0,0,0,0 NS = 0,0,0,0,0

TABLE 2: Average Irritation score (EXTOX/IC/ 008.Y13)

Animal No/ID	Extract	Irritation score	
		NS	CSO
1. 093♀	Control	0	0
	Test	0	0.26
2. 161 ♀	Control	0	0
	Test	0	0.33
3. 188♂	Control	0	0
	Test	0	0.20
Total mean score		NS: 0	CSO: 0.26

NS: Physiological Saline CSO: Cotton seed oil

TABLE 3: Grading system for Intracutaneous (Intradermal) reactions

Erythema	Score	Oedema	Score
No erythema	0	No oedema	0
Very slight erythema (barely perceptible)	1	Very slight oedema (barely perceptible)	1
Well-defined erythema	2	Well –defined oedema (edges of area well-defined by definite raising)	2
Moderate erythema	3	Moderate oedema (raised approximately 1mm)	3
Severe erythema (beet–redness to eschar formation preventing grading of erythema)	4	Severe oedema (raised more than 1mm and extending beyond exposure area)	4
Total possible score for irritation			8
Note: Other adverse changes at the injection sites shall be recorded and reported			

--End of Test Report: TRTOX 034.Y13----------

SREE CHITRA TIRUNAL INSTITUTE FOR MEDICAL SCIENCES AND TECHNOLOGY

(An Institute of National Importance under Govt. of India)

BIOMEDICAL TECHNOLOGY WING

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Phone: 91-471-234-0801, Fax: 91-471-234-1814, E-mail: csc@sctimst.ac.in

TEST REPORT (INTERNAL)

Study Title	:	Implantation in muscle of polymer, DTLAAS046-029 in albino Rabbit.
Work order/ Study number	:	WITOX D71.Y13
Test Material code	:	DTLAAS046-029
Toxicology Sample ID	:	TSTOX 024.Y13
Date of receipt of sample	:	25.04.13
Period of conduct of test	:	27.05.13 to 05.09.13
Study Report Number	:	TRTOX 053.Y13
Date	:	September 13, 2013

Test Facility
Toxicology Division, Biomedical Technology Wing
Sree Chitra Tirunal Institute for Medical Sciences and Technology
Thiruvananthapuram 695 012, Kerala, India.

Name of the Study Director: Dr. PV. Mohanan

Name and Address of the Pathologist
Dr. A Sabareeswaran
Histopathology Laboratory, Biomedical Technology Wing

Name and Address of Internal Customer
Mr. CV Muraleedharan, Device Testing Lab

Declaration

I hereby certify that this test certificate is for the sample received as per the above work order number.

Authorized Signatory: Dr. PV. Mohanan, SIC, Toxicology Division.
(Name & Designation)

GLP COMPLIANCE STATEMENT

Work Order/ Study No.	:	WITOX D71.Y13
Test material code	:	DTLAAS046-029
Study Title	:	Implantation in muscle of polymer, DTLAAS046-029 in albino Rabbits.

All the data presented in this report are original, correct and accurate to the best of my knowledge. I hereby attest that this study was conducted in accordance with the OECD principles of GLP.

The study was conducted to meet the requirements of 'ISO 10993-6: 2007 (E): Biological evaluation of medical devices - Part 6: Test for local effects after implantation: Annex C, Test method for implantation in muscle'.

Study Director

QUALITY ASSURANCE STATEMENT

This is to certify that to the best of signatories' knowledge, the above test report reflects the raw data as per the requirements of Good Laboratory Practices and Quality Assurance System as per ISO 17025 presently understood and practiced in the Biomedical Technology Wing, SCTIMST, Thiruvananthapuram.

Dr. P Ramesh
Quality Manager

PERSONNEL INVOLVED IN THE STUDY

Study director	:	Dr. PV. Mohanan
Study Pathologist	:	Dr. A Sabareeswaran
Study Personnel	:	Dr.V Gayathri
		Ms. Geetha C S
		Ms. Anju Mohan

SUMMARY

The study was designed to evaluate the response of muscle tissue following implantation of the test material, polymer, DTLAAS046-029 intended to be used for rotator cuff repair for shoulder reconstruction.

The study was conducted as per 'ISO 10993-6: 2007 (E): Biological evaluation of medical devices - Part 6: Test for local effects after implantation: Annex C, Test method for implantation in muscle' and in accordance with OECD principles of GLP.

The implantation procedure was carried out under clean and aseptic conditions. Rabbits were anaesthetized using Ketamine (80mg/kg body weight) + Xylazine (5mg/kg body weight). The skin of the anaesthetized rabbits were lightly swabbed with 70% alcohol and air dried. Five incisions were made on the skin and inserted five test implant materials (DTLAAS046-029) intramuscularly (Para vertebral muscle) along one side of the spine (right side), and about 25mm apart from each other. Similarly five control implant material (DTLAAS046-100C) was intramuscularly implanted in the contra lateral muscle (left side) of each rabbits. The incision was then closed using sterile sutures.

The study was conducted in 6 Rabbits (whose para vertebral muscle was sufficiently large), 3 animals each for 1 & 4 weeks. The body weight of the animals was not less than 2 kg.

At the end of each observation period, animals were sacrificed and collected the implants (both control and test) with surrounding tissues for histopathological analysis. Macroscopically there was no hemorrhage, encapsulation, discoloration, necrosis or infection at the implant sites at any of the observation period.

The general physical conditions of the experimental animals were normal. The increase in body weight and feed intake were normal and none of the animals showed any abnormality or behavioral changes during the experimental period.

The histopathological comprehensive report is enclosed (TRPAT 022.Y13). The test material is found to be non irritant at one week and four weeks post implantation.

Hence, the test material, polymer, DTLAAS046-029 (WITOX D71.Y13) meet the requirements of the test as per 'ISO 10993-6: 2007 (E): Biological evaluation of Medical devices: Part 6: Test for local effects after implantation: Annex C, Test method for implantation in muscle.

Introduction

The study, (WITOX D71.Y13) was designed for the evaluation of biocompatibility studies of polymer, DTLAAS046-029 in albino Rabbits

The study was conducted as per ISO 10993-6: 2007 (E): Biological evaluation of Medical devices Part 6: Test for local effects after implantation: Annex C, Test method for implantation in muscle and in accordance with OECD principles of GLP. The material, polymer, DTLAAS046-029 was used to evaluate the local effects after implantation in muscle at 1 and 4 weeks post implantation.

Identification of study

Title

Test for local effects after implantation: Test method for implantation in muscle of DTLAAS046-029 in albino Rabbits

Objective

The main objective of the study was to evaluate the biological response of muscle tissue to an implanted material after implantation.

Test item

The sponsor is responsible for the necessary evaluations of the test and control material's chemical purity, identity, stability and other required data. The details of the test substance are the following:

Name of test item	:	DTLAAS046-029
Preparation of test item	:	The material is cut from fabric, cleaned in ultrasonic cleaner, extracted in isopropyl alcohol and sterilized in ETO.
Nature of materials	:	Polymer
Physical Appearance	:	Strip
Manufacturer/ Source/ Supplied by	:	Device Testing Lab. BMT Wing, SCTIMST.
Batch/Lot Number	:	NA
Sterilization	:	ETO
Package	:	Packed.
Storage conditions	:	Room temperature
Handling	:	Under sterile conditions

Reference Item

Name of reference item	:	DTLAAS046-100C
Preparation	:	NA
Nature of materials	:	Polyester
Physical Appearance	:	Strip
Manufacturer/ Source/ Supplied by	:	Device Testing Lab, BMT Wing, SCTIMST.
Batch/Lot Number	:	NA
Sterilization	:	ETO
Package	:	Packed
Storage conditions	:	Room temperature
Handling	:	Under sterile conditions

Name of the study monitor	:	Nil

Study dates

Study Initiation	:	April 01, 2013
Initiation of experiment	:	May 27, 2013
Completion of experiment	:	September 05, 2013
Study completion	:	September 13, 2013

Test Method

ISO 10993-6: 2007 (E): Biological evaluation of Medical devices - Part 6: Test for local effects after implantation: Annex C, Test method for implantation in muscle.

Experimental Protocol

Test system Details

Name of Species	:	Rabbit
Strain of the Animal	:	Albino
Sex	:	Male/Female
Weight at start of treatment	:	Not less than 2.0 kg
Age	:	Adult
No. of Animals per period	:	3
Source	:	Division of Laboratory Animal Sciences, BMT Wing, SCTIMST
Room where test performed	:	Procedure Room, Toxicology

Justification of the Test System

ISO 10993-6: 2007 (E): Annex C recommends that the Paravertebral muscle of Rabbits is the preferred implantation sites. Alternatively the thigh muscles of Rabbits may be used. Historical data related to the pathological and biochemical parameters of this species are well documented.

Test system Identification

Individual animals were identified by tattoo number mark on animal's ear. In addition to this, each animal cage identified by labels and has study number, study name, group, sex, animal number(s), experiment initiation and experiment completion date.

Quarantine procedures

Since the animals were from our colony, there was no quarantine for these animals.

Acclimatization: Nil

Animal Husbandry and welfare

All animals were handled humanely, without making pain or distressing and with due care for their welfare. Animals care and management was in complying with the regulations of the Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA), Govt. of India. Institutional Animal Ethics Committee's approval (B form number: SCT/IAEC-041/DECEMBER/2012/79) was taken before initiating animal experiments.

Equipments/instruments used

Rabbit restrainer, disposable syringes, surgical instruments, sutures and anaesthetic agents.

Experimental animal room Sanitation

In addition to daily cleaning with disinfectant solution, the animal room was decontaminated before initiation of each experiment.

Environmental conditions
Animals were maintained in a controlled environment with a temperature 22±3°C, humidity of 30 -70 % and a light/dark cycle of 12 h. There will be a minimum of 15 fresh air changes per hour.

Housing Conditions
The animals were housed in a clean stainless steel fabricated cages.

Feed and water
The animals were provided with commercially available feed for Rabbit, and aqua guard filtered fresh drinking water *ad libitum* to the animals. The feed and water were analyzed every six months.

Fasting
Overnight fasted, prior to the test, water provided and 3-4 hours post medication. Thereafter feed and water was given *ad libitum*

Justification of the method
As per regulatory requirements the number and period of implantation has been chosen to characterize the toxicological profile of the test material to evaluate the biocompatibility of biomaterials intended for use in medical devices.

Test/Reference Item Administration

Delivery system	:	Material as such (Implantation in the para vertebral muscle)
Method	:	Direct application
Preparation for administration	:	Material as such
Frequency of administration	:	Single exposure
Number of samples	:	5 tests & 5 control samples/animal.

Experimental Procedure
Experimental design

Animal	:	Rabbit
No. of animals	:	6
Name of strain	:	New Zealand white
Sex of animals	:	Male/female
Body weight	:	Not less than 2 kg
Site of Implantation	:	Paravertebral Muscle
No. of samples	:	5 tests & 5 controls
Anesthesia	:	Ketamine: 80mg/kg, Xylazine: 5mg/kg (Intramuscular)

Duration of test procedure : 1 week and 4 weeks

Observations
Body weight
The body weight of the animals was recorded individually on the day of implantation and at the end of experiment (autopsy).

Explantation
At the end of each observation period, animals were sacrificed and collected the implants (both control and test) with surrounding tissues for histopathological analysis.

Histopathological evaluations were carried out on the following samples and the histopathological comprehensive report is enclosed

Comprehensive report: TRPAT 022.Y13

1 Week (EXTOX/IM/002.Y13)

Sample code *

TOX/IM/002.Y13 145 A1-A5
TOX/IM/002.Y13 145 B1-B4
TOX/IM/002.Y13 174 A1-A5
TOX/IM/002.Y13 174 B1-B5
TOX/IM/002.Y13 056 A1-A5
TOX/IM/002.Y13 056 B1-B3

4 Weeks (EXTOX/IM/003.Y13)

Sample code *

TOX/IM/003.Y13 097 A1-A5
TOX/IM/003.Y13 097 B1-B4
TOX/IM/003.Y13 160 A1-A4
TOX/IM/003.Y13 160 B1-B5
TOX/IM/003.Y13 098 A1-A5
TOX/IM/003.Y13 098 B1-B4

All 'A' are test materials (DTLAAS046-029) and all 'B' are control materials (DTLAAS046-100C).

Mortality: Nil

Evaluation Criteria
Evaluated the biological response by documenting the microscopic and histopathological responses as a function of time. Compared the responses of the test sample to the responses of the control sample or sham operated sites.

Results and conclusion
The general physical conditions of the experimental animals were normal. The increase in body weight and feed intake were normal and none of the animals showed any abnormality or behavioral changes during the experimental period. At the end of each observation period, animals were sacrificed and collected the implants (both control and test) with surrounding tissues for histopathological analysis. Macroscopically there was no hemorrhage, encapsulation, discoloration, necrosis or infection at the implant sites at any of the observation period.

The result of the intramuscular implantation in muscle was evaluated by comparing the control and test implants at equivalent locations. The histopathological comprehensive report is enclosed (TRPAT 022.Y13). The test material is found to be non irritant at one and four weeks post implantation.

Hence, the test material, DTLAAS046-029 (WITOX D71.Y13) meet the requirements of the test as per ISO 10993-6: 2007 (E): Biological evaluation of Medical devices: Part 6: Test for local effects after implantation: Annex C, Test method for implantation in muscle.

Study Plan Alteration (Amendment, Deviation): Nil

Good Laboratory Practices
The study was conducted in accordance to OECD principles of GLP and as per agreed protocol.

Archives
All the study related raw data together with the copy of final report was archived in the GLP archives of SCTIMST for five years. After the completion of this period sponsor consent will be sought to either extend the archive periods or return the archived material to the sponsor for the disposal of the material.

Report prepared by : Ms. Anju Mohan Anju

Report checked by : Dr. V Gayathri V. Gayathri

Test done, Verified and reported by : Dr. PV Mohanan

----------End of Test Report: TRTOX053.Y13----------

SREE CHITRA TIRUNAL INSTITUTE FOR MEDICAL SCIENCES AND TECHNOLOGY

(An Institute of National Importance under Govt. of India)

BIOMEDICAL TECHNOLOGY WING

Poojappura, Thiruvananthapuram 695 012 INDIA

Phone: 91-471-234-0801, Fax: 91-471-234-1814, E-mail: csc@sctimst.ac.in

TEST REPORT (INTERNAL)

Date: 05.09.2013 Report No: TRPAT 022.Y13 Total Number of pages: 10

Name of the laboratory: **HISTOPATHOLOGY**

1	Name of the test:	Gross and Histopathological Evaluation for Biocompatibility
2	Work order number:	WITOXD71.Y13, RQTOX 002.Y13
3.	Customer sample code:	See page 2
4.	Date of receipt of sample:	03.06.13, 24.06.13
5.	Date/Period of conduct of test:	June 2013 to September 2013
6.	Test method used:	ISO 10993 (6)
7.	Description of the sample:	Muscle with implant
8.	Details of specimen preparation (if applicable):	Specimen preserved in 10% buffered formalin
9.	Result (with units of measurement):	Report attached
10.	Name and address of the internal customer	Dr. P V Mohanan Scientist-in-charge Division of Toxicology

Declaration

I hereby certify that, this test certificate is for the sample received as per the above work order number.

11.	Authorised signatory Name and designation	Dr. A.Sabareeswaran Scientist-in-charge

05/09/13

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BIOMEDICAL TECHNOLOGY WING, POOJAPURA, TRIVANDRUM-695012

HISTOPATHOLOGY LABORATORY

DIVISION OF IMPLANT BIOLOGY

Test Report on Gross and Histopathological Evaluation for biocompatibility in subcutaneous tissue/muscle (ISO10993 - 6.)

1. **TRPAT No. : 022.Y13**

   1.1 SCPAT No: 006.Y13

   1.2 Sample Identification Details

Customer sample ID	TSPAT No
One week TOX / IM / 002 Y13 145 A1 to A5 TOX / IM / 002 Y13 145 B1 to B4 TOX / IM / 002 Y13 174 A1 to A5 TOX / IM / 002 Y13 174 B1 to B5 TOX / IM / 002 Y13 056 A1 to A5 TOX / IM / 002 Y13 056 B1 to B3	241 Y13 242 Y13 243 Y13 244 Y13 245 Y13 246 Y13
Four weeks TOX / IM / 003 Y13 097 A1 to A5 TOX / IM / 003 Y13 097 B1 to B4 TOX / IM / 003 Y13 160 A1 to A4 TOX / IM / 003 Y13 160 B1 to B5 TOX / IM / 003 Y13 098 A1 to A5 TOX / IM / 003 Y13 098 B1 to B4	249.Y13 250.Y13 251.Y13 252.Y13 253.Y13 254.Y13

2. Summary of Gross Pathology

(Based on observations recorded in RGPAT 008.000, Bk.21, pgs 78,79 and 82).

Received pieces of skeletal muscle. Sections were cut parallel to fascial surface till end of the implant was identified. As implant end appeared white fabric material in 241.Y13, 243.Y13, 245.Y13, 249.Y13, 251.Y13 and 253.Y13, cross sections were cut perpendicular to length of implant site along with implant. Central section from each muscle piece with implant was processed for histopathological evaluation.

In 242.Y13, 244.Y13, 246.Y13, 250.Y13, 252.Y13 and 254.Y13, the implant was a thin white fabric strip. Cross sections were cut perpendicular to length of implant site along with implant. Central section was processed for histopathological evaluation. The implant could not be identified grossly in 246.Y13B2 and 249.Y13A5.

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3. Summary of histopathological assessment: (Based on observations recorded in RGPAT 002.001, Bk.40, Pgs160 to 198 and Bk.41, Pgs1 to 13). Presented as table on page 5 to 10

Inflammatory cells – Polymorhphonuclear cells, Lymphocytes, Plasma cells and Macrophages - Grading based on the number and distribution of cells (0= 0 cells, 1=1-5 cells, 2=6-10 cells, 3 = heavy infiltration, 4 = packed cells, average of five fields at magnification 400x)
For Giant cells (0=0 cells, 1=1-2 cells, 2=3-5 cells, 3 = heavy infiltration, 4 = packed cells, average of five fields at magnification 400x)
Neovascularisation – Measurements under magnification of 400X (0= no capillaries, 1 = 1-3 capillaries, 2 = 4-7 capillaries, 3 = broad blood vessels, 4 = extensive vascularisation)
Fibrosis – Measurements under magnification of 400X determined by fibrous capsule thickness (0 = absent, 1 = <5µm, 2 = 6 – 15µm, 3 = 16 – 30µm, 4 = >30µ)
Necrosis – Grading (As determined by cell debris and inflammation: 0= not present,1 = minimally present, 2= mild degree, 3= moderate degree, 4= Severe degree)
Fatty infiltrate- Grading (As determined by amount of fat tissues: 0= not present, 1 = minimally present, 2= mild degree, 3= moderate degree, 4= Severe degree)
Tissue- in growth- Grading (As determined by amount of tissue ingrowth in case of porous and/or degradable materials: 0= not present, 1 = minimally present, 2= mild degree, 3= moderate degree, 4= Severe degree)

4. Mean Average Score:
Group total is sum of sample total I,II and III at one time period
Mean group average is group total divided by number of samples/ sections evaluated at one time period
Score*- Derived by deducting the mean group average of control from test. A negative difference is recorded as Zero.

For one week	**: 0**
For four weeks	**: 1.3**

5.Conclusion: Under the conditions of this study, the test sample was considered:

Non-irritant at one week.

Non-irritant at four weeks

(non-irritant (0.0 upto 2.9); slight irritant (3.0 upto 8.9); moderate irritant (9.0 upto 15.0); severe irritant (> 15))

At **one week** post implantation, degeneration and inflammation with macrophages is observed around the implant site in both groups. Moderate to severe degeneration is noted in test group and mild in control group. The inflammation is moderate in test group and mild to moderate in control group. Tissue ingrowth is observed moderately into the fabric, surrounding the loosely arranged monofilaments in the test group and mild in control group.

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Test Report No: TRPAT 022-13 Sign.I/c: Date: 05.09.13 Page sequence: 4 of 10

At **four weeks,** post implantation, repair with thin fibrous capsule formation is observed around the implant site in both groups with mild to moderate macrophage and lymphocyte infiltration. Foreign body type giant cells are seen moderately in test group and mild in control group. There is no evidence of necrosis. Severe tissue ingrowth is observed into the implant in test group and mild in

control group. The histological features in both groups are comparable; however the cellularity is more in test group.

Test report prepared / compiled by

DATE 05.09.13

Pathologist A. Sebareeswaran

Data transfer Verified by

Verified and recommended for issue:

DATE 05.09.13

Scientist-in-charge,
Histopathology Laboratory.

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Test Report No: TRPAT 022-Y13 Sign.I/c: Date: 05.09.13 Page sequence: 5 of 10

SCPAT - 006.Y13
ONE WEEK SAMPLE

	Test sample A				
TSPAT No:	241.Y13				
	A1	A2	A3	A4	A5
Number of sections	One	One	One	One	One
Section orientation	Cross section between both ends of implant/ implant site in skeletal muscle				
Implant orientation	Perpendicular	Perpendicular	Perpendicular	Perpendicular	Perpendicular
Cutting geometry	Irregular	Irregular	Irregular	Irregular	Irregular
Granuloma	Absent	Absent	Absent	Absent	Absent
Material debris	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable
Tissue in-growth	2	2	3	3	3
Inflammation					
Polymorphonuclear	1	1.2	1.2	1.2	1
Lymphocytes	0	0.8	0.2	0.4	0.4
Plasma cells	0	0	0	0.2	0
Macrophages	0.8	1.2	1.2	1	1.2
Giant cells	0	0	0	0	0
Necrosis	0	0	0	0	0
SUB-TOTAL	1.8	3.2	2.6	2.8	2.6
SUB-TOTAL X 2(a)	3.6	6.4	5.2	5.6	5.2
Neovascularisation	2	2	1	1	2
Fibrosis	3	3	3	3	3
Fatty infiltrate	0	0	0	0	0
SUB-TOTAL(b)	5	5	4	4	5
TOTAL(a+b)	8.6	11.4	9.2	9.6	10.2
SAMPLE TOTAL-I	49				

	Control sample B				
	242.Y13				
	B1	B2	B3	B4	
	One	One	One	One	
	Cross section between both ends of implant/ implant site in skeletal muscle				
	Perpendicular	Perpendicular	Perpendicular	Perpendicular	
	Irregular	Irregular	Irregular	Irregular	
	Absent	Absent	Absent	Absent	
	Not applicable	Not applicable	Not applicable	Not applicable	
	1	1	1	1	
	1.6	0.8	1	0.8	
	1	0.6	0.2	0.2	
	0	0	0	0	
	1.2	1.4	1.2	1	
	0.2	0.2	0	0.2	
	0	0	0	0	
	4	3	2.4	2.2	
	8	6	4.8	4.4	
	2	1	2	2	
	3	3	2	3	
	0	1	1	0	
	5	5	5	5	
	13	11	9.8	9.4	
	43.2				

cofrac

	Test sample A				
TSPAT No:	243.Y13				
	A1	A2	A3	A4	A5
Number of sections	One	One	One	One	One
Section orientation	Cross section between both ends of implant/ implant site in skeletal muscle				
Implant orientation	implant site could not be evaluated.	Perpendicular	Perpendicular	Perpendicular	implant site could not be evaluated.
Cutting geometry		Irregular	Irregular	Irregular	
Granuloma		Absent	Absent	Absent	
Material debris		Not applicable	Not applicable	Not applicable	
Tissue in-growth		3	3	3	
Inflammation					
Polymorphonuclear		1	1.2	1	
Lymphocytes		0.4	1	0.6	
Plasma cells		0	0	0	
Macrophages		1	1.4	1.2	
Giant cells		0	0.2	0	
Necrosis		0	0	0	
SUB-TOTAL		**2.4**	**3.8**	**2.8**	
SUB-TOTAL X 2(a)		**4.8**	**7.6**	**5.6**	
Neovascularisation		2	2	2	
Fibrosis		4	3	3	
Fatty infiltrate		0	0	0	
SUB-TOTAL(b)		**6**	**5**	**5**	
TOTAL(a+b)		**10.8**	**12.6**	**10.6**	
SAMPLE TOTAL- II			**34**		

Control sample B				
244.Y13				
B1	B2	B3	B4	B5
One	One	One	One	One
Cross section between both ends of implant/ implant site in skeletal muscle				
Perpendicular	Perpendicular	Perpendicular	Perpendicular	Perpendicular
Irregular	Irregular	Irregular	Irregular	Irregular
Absent	Absent	Absent	Absent	Absent
Not applicable	Not applicable	Not applicable	Not applicable	Not applicable
1	1	1	1	1
1.2	1	1.2	1	1
0.8	0.6	0.4	0.6	0.8
0	0	0	0	0
1.4	1	1	1	1.4
0.2	0.2	0	0	0.4
0	0	0	0	0
3.6	**2.8**	**2.6**	**2.6**	**3.6**
7.2	**5.6**	**5.2**	**5.2**	**7.2**
2	1	2	2	1
3	4	3	3	4
0	1	0	0	1
5	**6**	**5**	**5**	**6**
12.2	**11.6**	**10.2**	**10.2**	**13.2**
		57.4		

15/08/18

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Test Report No: TRPAT 022.Y13 Sign.I/c: Date: 05.09.13 Page sequence: 1 of 10

	Test sample A					Control sample B		
TSPAT No:	245.Y13					246.Y13		
	A1	A2	A3	A4	A5	B1	B3	
Number of sections	One	One	One	One	One	One	One	One
Section orientation	Cross section between both ends of implant/ implant site in skeletal muscle					Cross section between both ends of implant/ implant site in skeletal muscle		
Implant orientation	Perpendicular	Perpendicular	Perpendicular	Perpendicular		Perpendicular	Perpendicular	
Cutting geometry	Irregular	Irregular	Irregular	Irregular		Irregular	Irregular	
Granuloma	Absent	Absent	Absent	Absent		Absent	Absent	
Material debris	Not applicable	Not applicable	Not applicable	Not applicable		Not applicable	Not applicable	
Tissue in-growth	3	3	3	3		1	1	
Inflammation								
Polymorphonuclear	1.2	1.2	1.6	1.4		1	1.4	
Lymphocytes	0.4	0.4	1	0.4		0.8	0.2	
Plasma cells	0	0	0	0		0	0	
Macrophages	1	1	1	1		1.2	1	
Giant cells	0	0	0	0		0	0	
Necrosis	0	0	0	0		0	1	
SUB-TOTAL	2.6	2.6	3.6	2.8		3	3.6	
SUB-TOTAL X 2(a)	5.2	5.2	7.2	5.6		6	7.2	
Neovascularisation	2	2	1	1		2	1	
Fibrosis	3	3	2	2		3	2	
Fatty infiltrate	1	1	1	0		0	0	
SUB-TOTAL(b)	6	6	4	3		5	3	
TOTAL(a+b)	11.2	11.2	11.2	8.6		11	10.2	
SAMPLE TOTAL- III			42.2				21.2	
GROUP TOTAL (I+II+III)			125.2				121.8	
MEAN GROUP AVERAGE			10.43				11.07	
SCORE*	TEST - CONTROL			-0.6				

implant site could not be evaluated.

FOUR WEEKS SAMPLE

	Test sample A			
TSPAT No:	249.Y13			
	A1	A2	A3	A4
Number of sections	One	One	One	One
Section orientation	Cross section between both ends of implant/ implant site in skeletal muscle			
Implant orientation	Perpendicular	Perpendicular	Perpendicular	Perpendicular
Cutting geometry	Irregular	Irregular	Irregular	Irregular
Granuloma	Absent	Absent	Absent	Absent
Material debris	Not applicable	Not applicable	Not applicable	Not applicable
Tissue in-growth	4	4	4	4
Inflammation Polymorphonuclear	0	0	0	0.4
Lymphocytes	1	0.4	0.8	0.6
Plasma cells	0.4	0.4	0.2	0.6
Macrophages	1	1	0.8	1
Giant cells	1	1	0.8	1.2
Necrosis	0	0	0	0
SUB-TOTAL	3.4	2.8	2.6	3.8
SUB-TOTAL X 2(a)	6.8	5.6	5.2	7.6
Neovascularisation	1	1	1	1
Fibrosis	1	1	1	1
Fatty infiltrate	0	2	1	1
SUB-TOTAL(b)	2	4	3	3
TOTAL(a+b)	8.8	9.6	8.2	10.6
SAMPLE TOTAL-I	37.2			

Control sample B			
250.Y13			
B1	B2	B3	B4
One	One	One	One
Cross section between both ends of implant/ implant site in skeletal muscle			
Perpendicular	Perpendicular	Perpendicular	Perpendicular
Irregular	Irregular	Irregular	Irregular
Absent	Absent	Absent	Absent
Not applicable	Not applicable	Not applicable	Not applicable
2	2	2	2
0	0	0	0
0	0.8	1.2	0.8
0.2	0.3	0.6	0.2
1	0.8	1.2	1
0.2	0.4	0.4	0.4
0	0	0	0
1.4	2.3	3.4	2.4
2.8	4.6	6.8	4.8
1	1	2	1
1	1	2	2
0	0	1	1
2	2	5	4
4.8	6.6	11.8	8.8
32			

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Test Report No: TRPAT 022-13 Sign.I/c: Date: 05.09.13 Page sequence: 9 of 10

	Test sample A			
TSPAT No:	251.Y13			
	A1	A2	A3	A4
Number of sections	One	One	One	One
Section orientation	Cross section between both ends of implant/ implant site in skeletal muscle			
Implant orientation	Perpendicular	Perpendicular	Perpendicular	Perpendicular
Cutting geometry	Irregular	Irregular	Irregular	Irregular
Granuloma	Absent	Absent	Absent	Absent
Material debris	Not applicable	Not applicable	Not applicable	Not applicable
Tissue in-growth	4	3	4	4
Inflammation				
Polymorphonuclear	0	0	0	0
Lymphocytes	0.8	0.6	1	0.6
Plasma cells	0.2	0.4	0.6	0.2
Macrophages	1	1	1	1
Giant cells	0.6	1	1	0.8
Necrosis	0	0	0	0
SUB-TOTAL	2.6	3	3.6	2.6
SUB-TOTAL X 2(a)	5.2	6	7.2	5.2
Neovascularisation	1	1	1	2
Fibrosis	2	2	1	2
Fatty infiltrate	0	1	1	1
SUB-TOTAL(b)	3	4	3	5
TOTAL(a+b)	8.2	10	10.2	10.2
SAMPLE TOTAL- II	38.6			

	Control sample B				
	252.Y13				
	B1	B2	B3	B4	B5
	One	One	One	One	One
	Cross section between both ends of implant/ implant site in skeletal muscle				
	Perpendicular	Perpendicular	Perpendicular	Perpendicular	Perpendicular
	Irregular	Irregular	Irregular	Irregular	Irregular
	Absent	Absent	Absent	Absent	Absent
	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable
	2	2	2	2	2
	0	0	0	0	0
	0.8	1	0.6	0.8	0.6
	0.4	0.6	0.2	0.4	0.2
	1	1	1	1	0.8
	0.6	0.4	1.2	0.4	0.4
	0	0	0	0	0
	2.8	3	3	2.6	2
	5.6	6	6	5.2	4
	1	2	2	2	1
	2	2	2	2	2
	1	1	1	1	1
	4	5	5	5	4
	9.6	11	11	10.2	8
	49.8				

	Test sample A			
TSPAT No:	253.Y13			
	A1	A2	A3	A4
Number of sections	One	One	One	One
Section orientation	Cross section between both ends of implant/ implant site in skeletal muscle			
Implant orientation	Perpendicular	Perpendicular	Perpendicular	Perpendicular
Cutting geometry	Irregular	Irregular	Irregular	Irregular
Granuloma	Absent	Absent	Absent?	Absent
Material debris	Not applicable	Not applicable	Not applicable	Not applicable
Tissue in-growth	4	4	3	4
Inflammation				
Polymorphonuclear	0	0	0	0
Lymphocytes	0.8	0.8	0.8	0.4
Plasma cells	0.4	0.6	0.2	0.4
Macrophages	1	1.2	0.8	1
Giant cells	1	0.6	0.6	1
Necrosis	0	0	0	0
SUB-TOTAL	3.2	3.2	2.4	2.8
SUB-TOTAL X 2(a)	6.4	6.4	4.8	5.6
Neovascularisation	2	2	2	2
Fibrosis	2	2	2	2
Fatty infiltrate	1	2	0	1
SUB-TOTAL(b)	5	6	4	5
TOTAL(a+b)	11.4	12.4	8.8	10.6
SAMPLE TOTAL- III			43.2	
GROUP TOTAL (I+II+III)			119	
MEAN GROUP AVERAGE			9.92	
SCORE*	TEST - CONTROL		1.3	

Control sample B			
254.Y13			
B1	B2	B3	B4
One	One	One	One
Cross section between both ends of implant/ implant site in skeletal muscle			
Perpendicular	implant site could not be evaluated.	Perpendicular	Perpendicular
Irregular		Irregular	Irregular
Absent		Absent	Absent
Not applicable		Not applicable	Not applicable
2		2	2
0		0	0
0.2		0.6	1
0		0	0.2
1		0.8	1
0.6		0.8	0
0		0	0
1.8		2.2	2.2
3.6		4.4	4.4
1		1	1
1		1	1
1		1	1
3		3	3
6.6		7.4	7.4
		21.4	
		103.2	
		8.60	

SREE CHITRA TIRUNAL INSTITUTE FOR MEDICAL SCIENCES AND TECHNOLOGY

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TEST REPORT (INTERNAL)

Date: 06.11.2013 Report No: TR.TRU.132.Y13 Total Number of Pages: 3

Name of the laboratory: **THROMBOSIS RESEARCH UNIT**

1.	Name of the test:	*In-vitro* Hemocompatibility of materials
2.	Work order number:	WITRUD69.Y13
3.	Customer sample code:	DTLAAS 046 – 001,002 & 004.
4.	Date of receipt of sample:	08.04.2013
5.	Date/Period of conduct of test:	21.10.2013 & 22.10.2013.
6.	Test method used:	Exposure of materials with whole blood (WPTRU012) Estimation of activated compliment factors in human blood (WPTRU039), Percentage Hemolysis(WPTRU022), Partial thromboplastin time(WPTRU023), Light microscpoy(WPTRU028).
7.	Description of the sample:	Polymer
8.	Details of specimen preparation (if applicable):	See page 2
9.	Result (with units of measurement):	Attached
10.	Name and address of Internal Customer	C.V.Muraleedharan DTL

Declaration

I hereby certify that this test certificate is for the sample received as per the above work order number.

11.	Authorized Signatory, Name & Designation	Dr. Lissy K. Krishnan, Scientist G

Report on In -Vitro Hemocompatibility of Material

Work Order No: WITRUD69.Y13

Test sample codes: DTLAAS 046 – 001,002 & 004

Date of sample submission: 08.04.2013

Date of Testing: 21.10.2013 & 22.10.2013

Report No.: TR.TRU.132 .Y13

Date of Report: 06.11.2013

Reference: ISO 10993-4:2002 (E). Selection of tests for interaction of materials with blood.

Exposure of materials with blood: Blood from human volunteer was collected into the anticoagulant, CPD-A. Samples were placed in polystyrene plates and were immersed in PBS and agitated for 5 minutes. PBS was removed and 6 ml of blood was added, 1ml blood was taken immediately for initial analysis and remaining 5 ml blood was incubated with the samples for 30 min under agitation at 70 ± 5 rpm using an Environ shaker thermo stated at 35 ± 2°C (WPTRU012). Four empty polystyrene culture dishes were exposed with blood as reference.

Preparation of platelet poor plasma

The platelet poor plasma from blood was prepared as per WPTRU006. The blood sample was centrifuged at 4000rpm for 15 min and platelet poor plasma was aspirated.

Plasma hemoglobin:

The free hemoglobin liberated in to the plasma after exposure to sample was measured using Diode array Spectrophotometer as per WPTRU022.

Data: Plasma hemoglobin detected in the samples is given in table 1.

Percentage hemolysis:

The total hemoglobin in the whole blood samples were measured using automatic haematology analyzer (Sysmex-K 4500) as per WPTRU015. The free hemoglobin liberated in to the plasma after exposure to materials was measured using Diode array Spectrophotometer as per WPTRU022 and the percentage hemolysis was calculated using the formula (Free Hb /Total Hb) x 100.

Data: Percentage hemolysis detected in the plasma is given in table 2.

Complement Activation (C3a assay)

The Complement activation (C3a) was analyzed with commercially available ELISA kit (Quidel, USA) as per the method WPTRU039. Plasma samples were diluted 1:500 for performing the ELISA. Calibration curve was generated simultaneously, using the standard provided by the reagent manufacturer, as per the instructions. For measurement of absorbance BioRad iMark micro plate reader was used. The calculations were done using software Excel.

Data: C3a concentration detected in samples before (initial) and after (final) exposure are given in table 3.

Plasma coagulation (PTT)

Partial thromboplastin time in each sample was detected using a reagent kit obtained from Diagnostica Stago (France) on Start 4, coagulation analyzer as per WPTRU023.

Data: PTT detected in initial and final samples are given in table 4.

Light microscopy

The devices after 30 min exposure were rinsed thoroughly in PBS and were fixed with 0.2 % gluteraldehyde for 1 h. They were then developed with Giemsa stain as per WPTRU028. Samples were viewed under light microscope to detect cell adhesion.

Note. Microscopy was not feasible. Material adsorbed stain and cells could not be resolved.

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TEST REPORT (INTERNAL)

Study Title	:	Guinea pig Maximization test (GPMT) of Physiological saline extract of polymer, DTLAAS046-054
Work order/Study number	:	WITOX D72.Y13
Test material code	:	DTLAAS046-054
Toxicology sample ID	:	TSTOX021.Y13
Date of receipt of sample	:	10.04.13
Period of conduct of test	:	24.04.2013 to 24.05.2013
Study Report Number	:	TRTOX035.Y13
Date	:	May 24, 2013

Test Facility

Toxicology Division
Biomedical Technology Wing,
Sree Chitra Tirunal Institute for Medical Sciences and Technology,
Thiruvananthapuram 695 012, Kerala, India

Name and Address of the Study Director

Dr. PV. Mohanan
Scientist In Charge, Toxicology Division
Biomedical Technology Wing,
Sree Chitra Tirunal Institute for Medical Sciences and Technology,
Thiruvananthapuram 695 012, Kerala, India

Name and Address of Internal Customer

Mr. CV Muraleedharan
Device Testing Lab

Authorized Signatory : Dr. P. V. Mohanan, SIC, Toxicology Division.
(Name & Designation)

GLP COMPLIANCE STATEMENT

Work order/Study No.	:	WITOX D72.Y13
Test material code	:	DTLAAS046-054
Study Title	:	Guinea pig Maximization test (GPMT) of Physiological saline extract of polymer, DTLAAS046-054

All the data presented in this report are original, correct and accurate to the best of my knowledge. I hereby attest that this study was conducted in compliance with the OECD Principles of GLP.

The study was conducted to meet the requirements of the 'ISO 10993-10: 2010 (E), Biological evaluation of medical devices - Part 10: Test for irritation and skin sensitization, Clause 7.5: Guinea pig Maximization test (GPMT).

Study Director

QUALITY ASSURANCE STATEMENT

This is to certify that to the best of signatories' knowledge, the above test report reflects the raw data as per the requirements of Good Laboratory Practices and Quality Assurance System as per ISO 17025 presently understood and practiced in the Biomedical Technology Wing, SCTIMST, Thiruvananthapuram.

Dr. P Ramesh
Quality Manager

PERSONNEL INVOLVED IN THE STUDY

Study Director	:	Dr. P V. Mohanan
Study Personnel	:	Ms. Geetha. CS
		Dr. Gayathri. V

SUMMARY

This study was designed to determine the skin sensitization potential in Guinea pigs by Maximization test (GPMT) of the Physiological saline extract of polymer, DTLAAS046-054, intended to be used as implant used for rotator cuff repair for shoulder reconstruction.

The study was conducted in accordance with 'ISO 10993-10: 2010 (E): Biological evaluation of medical devices - Part 10: Test for irritation and skin sensitization. Clause: 7.5: Guinea pig Maximization test (GPMT) and in compliance with OECD principles of GLP.

In this study there were 15 Guinea pigs for the extract (10 for test and 5 for control). The body weight range of the animals was 300-500g.

The Physiological saline (PS) extract of test material (DTLAAS046-054) and control (PS alone) was intradermally injected and after seven days it was topically applied. Challenge test was carried out after fourteen days on all the animals. The appearance of the challenge skin sites of test and control animals were observed at 24h, 48h and 72h after removal of dressings and patches. The skin reactions for erythema and oedema were scored and recorded the numerical grading as per ISO 10993-10: 2010 (E).

The result of the study indicated that the Physiological saline extract of the test material and control treated animals did not show any adverse skin reaction during the induction or challenge period and confirmed that the Physiological saline extract of the test material is non irritant at the laboratory conditions simulated.

Hence, the Physiological saline extract of the test material, polymer, DTLAAS046-054 (WITOX D72.Y13) meet the requirements of the test as per 'ISO 10993-10: 2010 (E), Biological evaluation of medical devices - Part 10: Test for irritation and skin sensitization, Clause 7.5: Guinea pig Maximization test (GPMT).

Introduction

The study, WITOX D72.Y13 was designed for the Guinea pig Maximization test (GPMT) of Physiological saline (PS) extract of polymer, DTLAAS046-054.

The study was conducted in accordance with 'ISO 10993-10: 2010 (E): Biological evaluation of medical devices -- Part 10: Test for irritation and skin sensitization, Clause 7.5: Guinea pig Maximization test (GPMT).

The Physiological saline extract of DTLAAS046-054 was used to evaluate skin sensitization elicited after induction and challenge phase of test in Albino Guinea pigs.

Identification of study

Title

Guinea pig Maximization test with Physiological saline extract of polymer, DTLAAS046-054.

Objective

The objective of the study is to evaluate the skin sensitization potential of the Physiological saline extract of polymer, DTLAAS046-054 on albino Guinea pigs by Maximization test.

Test item details

The sponsor is responsible for necessary evaluations of the test substance purity, identity, stability and other required data. The details of the test substance are the following:

Name of test item	:	DTLAAS046-054
Preparation of test item	:	The material is cut from fabric, cleaned in ultrasonic cleaner, extracted in isopropyl alcohol and sterilized in ETO
Nature of materials	:	Polymer
Physical Appearance	:	Fabric
Manufacturer/Source of test item /Supplied by	:	Device Testing Lab, BMT Wing, SCTIMST
Batch/Lot No/catalogue/formulation/ date of manufacture	:	NA
Trade Name	:	NA
Sterilization	:	ETO
Package	:	Packed
Storage conditions	:	Room temperature
Handling	:	Aseptic conditions

Reference Item details

Name of reference item	:	Physiological saline
Preparation	:	Commercially available
Nature of materials	:	Liquid
Physical Appearance	:	Clear solution
Manufacturer/Source	:	M/s Parentral drugs Ltd
Supplied by	:	Sreeja Medicals,Trivandrum
Batch/Lot Number	:	2C-292 September, 2012
Sterilization	:	Sterile
Package	:	Bottle
Storage conditions	:	Room Temperature

Handling	:	Aseptic condition
Name of the study monitor	:	Nil

Study dates

Study Initiation	:	April 01, 2013
Initiation of experiment	:	April 24, 2013
Completion of experiment	:	May 24, 2013
Study completion	:	May 24, 2013

Test Methods

ISO 10993-10: 2010 (E), Biological evaluation of medical devices: Test for irritation and skin sensitization, Clause 7.5: Guinea pig Maximization test.

Experimental Protocol

Test system details

Name of Species	:	Guinea pigs
Strain of the Animal	:	Albino
Sex	:	Male/Female
Weight at start of treatment	:	300g to 500g
Age	:	Adult
Animal preparation	:	Healthy, thin skinned Guinea pigs, whose fur are clipped closely on either side of the spinal column (dorsal side) and ensure that the skin is free of mechanical irritation or trauma.
No. of Animals per extract	:	15 (10 for test and 5 for control)
Source of supply	:	Laboratory Animal Sciences, BMT Wing, SCTIMST
Room where test performed	:	Experimental Animal room

Justification of the Test System

ISO 10993-10: 2010 (E), Clause 7.5 recommends the usage of Guinea pigs for skin sensitization studies by topical application and also Guinea pigs are reported to be a suitable model for pre-clinical safety evaluation. Historically guinea pigs have been shown to be sensitive to the sensitization potential of a variety of compounds including chemicals, pesticides and drugs. Handling and availability of this animal is easy.

Test system Identification

Individual animals were identified with picric acid mark. In addition to this, each animal cage was identified by labels having experiment number, group, animal number(s), sex, experiment initiation and experiment completion date.

Quarantine procedures

Since the animals were from our colony, there was no quarantine for these animals.

Acclimatization

The animals were acclimatized for 6 days before initiation of experiment.

Animal Husbandry and welfare

All animals were handled humanely, without making pain or distressing and with due care for their welfare. Animals care and management was in comply with the regulations of the Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA), Govt., of India. Institutional

Animal Ethics Committee's approval was taken before initiating animal experiments (Approval No: SCT/IAEC-035/DECEMBER/2012/79)

Equipments/instruments used
Disposable syringe (batch no. 237014G31), laminar bench (EQTOX019) Incubator shaker (EQTOX628), pH meter (EQTOX074).

Experimental animal room Sanitation
In addition to daily cleaning with disinfectant solution, the animal room was decontaminated before initiation of each experiment.

Environmental conditions
Animals were maintained in a controlled environment with a temperature 22±3°C, humidity of 30 -70 % and a light/dark cycle of 12 h. There will be a minimum of 15 fresh air changes per hour.

Housing Conditions
The animals were housed in a clean stainless steel fabricated cage. Each animal was individually caged.

Feed and water
The animals were provided with commercially available feed for Guinea pig and aqua guard filtered fresh drinking water *ad libitum*. The feed and water was analyzed every six months.

Fasting
Pre or post medication fasting not required.

Justification of the method
As per regulatory requirements and also the dose routes has been chosen to characterize the toxicological profile of the test extract. To evaluate the toxic end points after the application.

Test/Reference Item Administration

Delivery system	:	Extract injection and topical application.
Method	:	Intradermal injection and Topical application.
Preparation for administration	:	Physiological saline extract as per ISO 10993 - 12: 2007 (E): Sample preparation and Reference materials.
Frequency of administration	:	Single Intra dermal injection (0.1 mL/2 sites).Topical application after seven days and Challenge phase after fourteen days.
Amount of extract required	:	20mL

Experimental Procedure
Extraction of material

Test item	:	DTLAAS046-054	
		Test	Control
Surface area(cm^2)/weight of sample(gm)	:	60 cm^2	-
Extraction temperature	:	50°C ± 2°C	50°C ± 2°C
Extraction media	:	Physiological saline	Physiological saline
Volume of media	:	20mL+ 4mL absorption capacity	20mL
Period of extraction	:	72h ±2h	72h ±2h
No. of replicates	:	One	One
Speed of agitation	:	50 rpm	50 rpm
Nature of extract		Clear	Clear

Experimental design

Details	Extraction media: Physiological saline	
	Test	Control
No. of animals	10	5
Name of species	Guinea pig	
Name of strain	Albino	
Sex of animals	Male/female	
Body weight	300-500g	
Route of application	Intradermal injection & Topical application	
Dosage of application	0.1mL/site x 2 for induction phase Undiluted extract for Topical application	

Duration of test procedure

Intra-dermal Induction phase:

Three sites per animal. Make a pair of 0.1ml intradermal injections.

Site 1: 0.1mL from 50: 50 volume ratio, stable emulsion of Freund's complete adjuvant mixed with extraction media (all 15 animals).

Site 2: 0.1mL of undiluted extract for test animals and extraction media for control animals.

Site 3: 0.1ml from the mixture of site 1 and 2 in 50:50 volume ratio.

Topical Induction phase:

Seven days (± 1day) after intradermal injection, the test and control extracts were topically applied to intrascapular region of each Guinea pig by saturating a patch of absorbent gauze ($8cm^2$) with the test/control extracts. Forty eight hours later, the dressings and patches were removed. Twenty four hours prior to topical application, the sites were treated with 10% sodium lauryl sulfate.

Challenge phase:

Fourteen days after topical application, the test and control animals were challenged with test material extract. The patches and dressings were removed after 24h.

Observations

The appearance of the challenge skin sites of test and control animals were observed at 24h, 48h and 72h after removal of dressings and patches. The skin reactions for erythema and oedema were scored and recorded the numerical grading as per ISO 10993-10: 2010(E) (Table 1).

Body weight

The body weight of the animals was recorded individually on the initiation of acclimatization, day of treatment and before challenge test.

Mortality

No mortality or morbidity was observed.

Evaluation Criteria

Grades of 1 or greater in the test group generally indicate sensitization, provided grades of less than 1 are seen on control animals. If grades 1 or greater noted on control animals, then the reactions of test animals that exceed the most severe control reaction are presumed to be due to sensitization. If the response is equivocal, a rechallenge is recommended to confirm the results from the first challenge.

Results and conclusion

The result of Guinea pig Maximization test was evaluated on the basis of the potential of the test material extract to elicit skin sensitization (erythema /oedema).

The numerical grading:

	Erythema	Oedema
	NS	NS
Control	0	0
Material extract	0	0

The Physiological saline extract of test material and control treated animals did not elicit any skin sensitization in Guinea pigs, which confirmed that the Physiological saline extract of the test material is non irritant at the laboratory conditions simulated (Table 2).

Hence, the Physiological saline extract of the test material, polymer, DTLAAS046-054 (WITOX D72.Y13) meet the requirements of the test as per ISO 10993-10: 2010 (E), Biological evaluation of medical devices -- Part 10: Test for irritation and skin sensitization, Clause: 7.5: Guinea pig Maximization test (GPMT).

Study Plan Alteration (Amendment, Deviation): Nil

Good Laboratory Practices

The study was conducted in accordance to OECD principles of GLP and as per agreed protocol.

Archives

All the study related raw data together with the copy of final report was archived in the GLP archives of SCTIMST for five years. After the completion of this period sponsor consent will be sought to either extend the archive periods or return the archived material to the sponsor for the disposal of the material.

Test done and Report prepared by : Ms. Geetha CS

Report checked by : Dr. Gayathri V

Verified and Reported by : Dr. PV. Mohanan

Table 1: Magnusson and Kligman Scale

Patch test reaction	Grading Scale
No visible change	0
Discrete or patchy erythema	1
Moderate and confluent erythema	2
Intense erythema and swelling	3

Table 2: Observation

EXPT. NO: EXTOX/SZ/001.Y13 DATE: 22.05.13 TO 24.05.13

Animal No.	Group	Skin reaction					
		Erythema			Oedema		
		24h	48h	72h	24h	48h	72h
1	Test	0	0	0	0	0	0
2	Test	0	0	0	0	0	0
3	Test	0	0	0	0	0	0
4	Test	0	0	0	0	0	0
5	Test	0	0	0	0	0	0
6	Test	0	0	0	0	0	0
7	Test	0	0	0	0	0	0
8	Test	0	0	0	0	0	0
9	Test	0	0	0	0	0	0
10	Test	0	0	0	0	0	0
11	Control	0	0	0	0	0	0
12	Control	0	0	0	0	0	0
13	Control	0	0	0	0	0	0
14	Control	0	0	0	0	0	0
15	Control	0	0	0	0	0	0

----------End of Test Report: TRTOX035.Y13----------