Technical Specification for "Development of a large animal model and testing the safety and performance of *pelvic floor mesh*"

S.No	Technical Specification	Compliance (Yes/No)	Additional Information if Any
1	**About the work:** Development of an animal model and testing the safety and performance of *pelvic floor mesh*		
2	**Overall study objective:** a) Developing a suitable large animal (pig) model that mimics human female pelvic organ prolapse b) To evaluate safety and performance of ***pelvic floor mesh***, a urogynecologic polycaprolactone surgical mesh in a female ovariectomized pig model.		
3	**Outcome Measure:**		
3.1	**Development of an animal model:** The work is broadly divided into three segments: a) Establishment of two surgical protocol in ovariectomised Large White or the "Yorkshire" (British breed of domestic weighing 90±10 kgs) pig model b) One month experiment and analysis (n=2), c) Three- month experiment and analysis (n=2).		
3.2	**Establishment of surgical protocol:** The study involves mimicking the menopausal female pelvic organ prolapse in ovariectomised Large White or the "Yorkshire" pig.		
3.2.1	**One month study:** The one-month study is required to try out two different surgical protocol and check the performance of ***Pelvic floor mesh*** for period of one month. The best surgical model is chosen which is then utilised for three-month experiments in ovariectomised female pelvic organ prolapse in Large White or the "Yorkshire" pig model. a) For one month experiment, the number of ovariectomised Large White or the "Yorkshire" pig model required is two only. Non-cyclic gilts that are around 150±20 days of age, weighing 90±10 kgs and that have just reached puberty will be chosen for this study. b) For the first ovariectomised Large White or the "Yorkshire" pig, the uterosacral ligament will be identified and partially ligated towards the cervix-vagina junction. ***Pelvic floor mesh*** supplied by IITB will be inserted below each uterine horn. The mesh arms will be sutured to the sacrum end of the uterosacral ligament using nonabsorbable polypropylene suture. In this case, the mesh will hold each uterine horn like a hammock in the absence of the uterosacral ligament support. This protocol may be revised based on mutual discussion. c) For the second ovariectomised Large White or the "Yorkshire" pig, ***Pelvic floor mesh*** will be inserted below each uterine horn. The arms of ***Pelvic floor mesh*** will be sutured to the sacrum end of the uterosacral ligament using nonabsorbable polypropylene suture. In this case, ***Pelvic floor mesh*** will hold the uterine horns like a hammock in the presence of the uterosacral ligament support. This protocol may be revised based on mutual discussion. d) At the end of the study, the mesh tissue complex is explanted and assessed, as mentioned in section D. Three small portions of the mesh tissue complex of size 5 cm x 1 cm will be sent back to IITB for mechanical property analysis.		
3.2.2	**Three month study:** Three-month experiments on ovariectomised Large White or the "Yorkshire" pig model with analysis: a) Based on the above analysis, a suitable experiment is chosen for further studies. b) Two animals (ovariectomised pig) at this time point will be used for this study. c) At the end of the study, all the assessment is performed as per section D. Additionally, H&E staining of all the major organs like liver, kidney, heart, adrenal, spleen, muscle, brain, lungs and bronchi will be performed.		

d) Three small portions of the mesh tissue complex of size 5 cm x 1 cm will be sent to IITB for mechanical property analysis.
e) This protocol may be revised based on mutual discussion.

3.3 **Evaluation of safety and performance in the animal model:**

The outcome of success is broadly determined by the safety and performance of ***Pelvic floor mesh.***

a) Outcomes for safety will be measured through inflammatory parameters, blood markers, and tissue histopathology.
b) Outcomes for performance will be measured as per the following (i) higher collagen production, (ii) negligible inflammation, (iii) uniform fibrosis throughout and (iv) load-bearing ability of pelvic mesh.

3.3.1 **Parameters of assessment**

The parameters of assessment at the end of the study can be broadly classified into:

a) Macroscopic anatomical examination,
b) Histopathology examination,
c) Immunohistochemistry assessment,
d) Gene expression assessment,
e) Assessment of haematology parameters,
f) Blood biochemistry, and
g) Coagulation parameters.

3.3.1.1 **Macroscopic anatomical evaluation**

a) Angiogenesis, Encapsulation, haematoma, contraction, separation, exposure, extrusion, perforation, dehiscence, fluid collection and infection.
b) The results will be graded on a scale of 0 to 4 (0-none, 1-minimal, 2-mild, 3-moderate and 4-severe) and the inference for each scale will be mentioned

3.3.1.2 **Histopathology Examination**

Histopathology examination of mesh tissue explant and major organs such as liver, kidney, heart, adrenal, spleen, muscle, brain, lung and bronchi.

a) Look for Identification and grading of fibrosis, giant cells, fatty infiltration, lymphocytes, macrophages, necrosis, neovascularisation, plasma cells and polymorphonuclear cells), Masson trichrome staining, Sirius red staining, hydroxyproline assay, and immunohistochemistry of CD34, α-SMA, CD45, M1 and M2.
b) The histology images will have to be taken in optical and/ or stereo microscope and the grading will have to be done as per table 1 and 2.

Table 1. Histological evaluation scoring system-cell type/response

Cell type / Responses	**Scores**				
Polymorphonuclear Cells	**0**	**1**	**2**	**3**	**4**
Lymphocytes	Nil	Rare, 1-5/phf*	5-10/phf	Heavy Infiltrate	Packed
Plasma Cells	Nil	Rare, 1-5/phf*	5-10/phf	Heavy Infiltrate	Packed
Macrophages	Nil	Rare, 1-5/phf*	5-10/phf	Heavy Infiltrate	Packed
Giant Cells	Nil	Rare, 1-2/phf	3-5/phf	Heavy Infiltrate	Sheets
Necrosis	Nil	Minimal	Mild	Moderate	Severe
*phf = per high powered (400 x) field					

Table 2. Healing responses

Tissue responses	**Scores**				
	0	**1**	**2**	**3**	**4**
Neovascularization	0	Minimal capillary, proliferation, focal, 1-3 buds	Groups of 4-7 capillaries with supporting fibroblastic structures	Broad band of capillaries with supporting structures	Extensive band of capillaries with supporting fibroblastic structures

	Fibrosis	0	Narrow band	Moderately thick band	Thick band	Extensive band		
	Fatty infiltrate	0	Minimal amount of fat associated with fibrosis	Several layers of fat and fibrosis	Elongated and broad accumulation of fat cells about the implant site	Extensive fat completely surrounding the implant		
	The images have to be graded for fibrosis, bridging and collagen on a scale of 0 to 4 (0 = None, 1 = Minimal, 2 = Mild, 3 = Moderate and 4 = Severe) and the inference for each scale has to be mentioned individually for all the parameters along with annotations. In the final report, images of minimum 3 different magnification per mesh tissue explant has to be reported.							
3.3.1.3	**Gene expression assessment** a) The following genes have to be quantified on the tissue in contact with mesh GAPDH, TNF-α, IL6, IL10, TGF-β, collagen I and collagen III.							
3.3.1.4	**Assessment of haematology** Assessment of haematology parameters such as red blood cell (RBC) count, white blood cell (WBC) – total and differential count, platelet count, hemoglobin (Hb), and hematocrit.							
3.3.1.5	**Assessment of blood biochemistry** a) Measurement of liver function: Alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, proteins (total proteins, albumin (ALB), globulin (GLB), glucose, cholesterol and triglyceride. b) Measurement of pancreas function: Amylase (AMYL) and lipase (LIP)							
3.3.1.6	**Electrolytes** Electrolytes such as (calcium (Ca), magnesium (Mg), chloride (Cl), potassium (K), sodium (Na), and phosphorus (PHOS)							
3.3.1.7	**Assessment of coagulation parameters** Measurement of activated partial thromboplastin time (aPTT), prothrombin time (PT), thrombin time (TT), fibrinogen level, D-Dimer (DD), and antithrombin III (ATIII).							
4	**Investigational product** a) Preclinical Contract Research Organization (**CRO**) shall use the investigational product for the sole purpose of conducting the Study. CRO shall handle, use, and store, only at the CRO in accordance with all applicable laws and requirements. b) At all times, CRO shall maintain complete and accurate written records regarding the inventory, use, containment and disposition of the used investigational product. c) CRO shall use investigational product only to conduct the Study and shall not chemically, physically or otherwise modify. d) At the conclusion of the Study, or upon the earlier termination of this Agreement, CRO shall return or disposition of any remaining unused quantities of investigational product							
5	**Animal ethics committee approval** The CRO shall be responsible for submission and obtaining approval of the Study and study-related documents from the institutional Animal Ethics Committee, prior to commencing the Study.							
6.	**Record keeping, meetings and reports** a) CRO shall prepare, maintain, and retain complete, current, accurate, organized, and legible documents, and other written or electronic records, accounts, notes, reports, databases, and data relating to the Study. b) CRO shall keep such records in a safe and secure manner for at least three (3) years following the latter of expiration or termination of this Agreement. c) CRO shall update Sponsor (IIT Bombay) on the progress of the Study on a regular basis in teleconferences and other forums as agreed upon by the Parties. d) CRO shall provide Sponsor a copy of signed and dated Curriculum Vitae of principal employee conducting/ coordinating the Study.							
7	**Publication**							

	a) The Parties agree that all publications under this Agreement shall adhere to generally accepted principles of authorship, and the Parties shall refer to the standards of the International Committee of Medical Journal Editors to resolve any dispute over authorship. b) The parties acknowledge that the Sponsor shall retain ownership of copies of source data that result from this Study. Sponsor accepts the obligation to facilitate publication of medically critical data in a timely, objective, accurate, and balanced manner, regardless of the outcome of the evaluation. c) All information arising out of this study will be the proprietary of the Sponsor. The CRO may NOT publish, present and use for instruction and research any results arising out of its conduct of the study.		
8	**Data and/or report submission** The data and/ or report of all the studies have to be reported within 3 weeks after the end of one month and three month study.		
9	**Payment Terms** 1) 50% Payment upon IEC approval 2) 40 % Upon first Draft 3) 10 % Upon work completion		