# Collection, Processing and Storage of Prostate Specimens for Tissue Banking

**CONTENTS**

## RISK ASSESSMENT

### Hazards:

1. Risk of inhalation and splashes from formalin.
2. Risk of cuts from sharps/scalpel blades.
3. Risk of infection from unfixed (Fresh) specimens.
4. Burning due to contact with liquid nitrogen.

### Safety Measures:

1. Personal protective equipment i.e. white coat, gloves and eye protection must be worn at all times.
2. Use forceps to remove samples from liquid nitrogen.
3. Take care to prevent spillage of liquid nitrogen. After use the container should be left open in fume hood to allow evaporation.
4. Refer to Health and Safety Manual in main laboratory, relevant documents are LP-PAT-LIQNIT and LP-PAT-HIRISK

**See:** **PRA-HIS-TBProcess.**
**HAZARD RATING: 16-40 Significant Risk**

**BRA-HIS-FreshTissue**
**HAZARD RATING: 16-40 Significant Risk**

**CRA-HIS-RNALater**
**HAZARD RATING: 6-15 Low Risk**

# 0 INTRODUCTION

## *0.1 Scope and purpose*

The purpose of tissue banking is to provide a broad range of tumour samples and adjacent normal tissue samples for research purposes when needed. This document outlines the steps involved in the collection, processing and storage of Prostate tissue samples for the RCSI/Beaumont tissue bank.

## *0.2 Responsibility*

The Medical Laboratory Assistant assigned to tissue banking or a Senior Medical Scientist.

## *0.3 References*

1. THE CNIO (Centro Nacional de Investigaciones Oncológicas) tumour bank network (http://www.cnio.es/ing/programas/progTumor01.asp & http://www.cnio.es/ing/programas/progTumor11.asp)

2. The NCI Cooperative Human Tissue Network (http://www.chtn.ims.nci.nih.gov/)

## *0.4 Definitions*

PPE – Personal Protective Equipment

## *0.5 Related documents*

DD-PAT-H&S Manual: Department of Pathology Health & Safety Manual
CP-HIS-TBDatabase: Use and maintenance of the RCSI Tissue Bank Database.
LF-HIS-TBDatabase: Tissue Bank Database
QP-HIS-TBAudit: Internal Auditing of the RCSI Tissue Bank
QF-HIS-TBAudit: Internal Audit record of the RCSI Tissue Bank
LF-HIS-TBProst: Tissue Banking – Prostate Details
LF-HIS-TBProstCut: Dissection of Fresh Prostate for Tissue Banking
CP-HIS-FWUL3: Use and management of "FreezerworksUL3" for RCSI Tissue Banking

# 1.0 PROCEDURE AND METHODS

## *1.1 Collection of Tissue Sample*

1.1.1 A fresh Prostate sample is removed from a patient in the operating theatre.

1.1.2 The Research Nurse (currently Maureen) is responsible for the Prostate collection from theatre and will bring the specimen/tissue (Fresh) to the Beaumont histology laboratory.

1.1.3 The person assigned to Tissue Banking (currently Ciara Heeney) is paged (Bleep 687) by the research nurse just before leaving theatre.

1.1.4 The Research Nurse is then contacted to confirm a Fresh tissue sample is ready and on route to the Beaumont Histology laboratory.

1.1.5 The Research Nurse must only pass on the Prostate specimen to either the person assigned to the tissue banking rota or a nominated Senior Medical Scientist.

## *1.2 Processing of Tissue Samples*

1.2.1 All Prostate tissue bank sample information must be recorded on form LF-HIS-TBProst (Tissue Banking – Prostate Details).

1.2.2 PPE (gloves can be found in Histology Cut-up room/Beaumont) must be worn when processing a tissue sample.

1.2.3 The following items are required to process the sample and should be collected from the RCSI Specimen Processing room:

- Laboratory form LF-HIS-TBProct
- 4 - 8 x 1.8ml cryovials
- Cassettes
- 10% Buffered Formalin
- Allprotect™
- RNA*later*®
- Plastic 3ml pipettes
- Fine permanent marker
- RNase*Zap*® wipes
- Liquid Nitrogen dewar
- Forceps
- Disposable Blades
- Camera

*1.2.4* If any extra processing materials are needed, there is an allocated bucket in the Beaumont histology lab (labelled 'RCSI/Beaumont Tissue Banking') containing extra cryovials tubes, coloured disks for the cryovial lids, 3ml disposable pipettes, RNA*later*®, *Allprotect*™, RNaseZap® wipes, DEPC water, tissue paper, disposable blades, cassettes and a fine permanent marker.

1.2.5 There are specific colour coded tubes used for each type of tissue. The following are the tissues and matching colours currently in use:

Colon = Blue
Colonic Biopsies = Yellow
Breast = Green
Skin = Red
Others = White
Prostate = Pink

1.2.6 On receipt of the specimen from the Research Nurse ensure it is labelled with a Beaumont Hospital Surgical number. If not, assign the next available surgical number from the roll of numbers in the histopathology/specimen reception room. Place one sticker on the top of the specimen container, one on the side of the container and one on the patient requisition card (i.e. the card accompanying the specimen).

1.2.7 Transport the specimen into the cut-up area where a Pathologist (currently Elaine Kay, Bleep: 327, mobile: 087-2529455) must dissect it using aseptic technique.

1.2.8 If there is no Pathologist in the cut-up room or in the Pathology Registrar's room, ask a member of the Beaumont histopathology team to bleep the Pathologist on cut-up duty for that week.

1.2.9 Specimen dissection to be carried out in the biological fume hood. Clean down the cut-up board, weighing scales, scalpel handle and sampling forceps with an RNase Zap wipe followed by a tissue soaked in distilled water. Place a new size 22 blade on the scalpel handle. Leave a disposable ruler out to allow the Pathologist to take measurements if needed.

1.2.10 Label four to eight cryovials with the surgical number assigned to the patient.

1.2.11 Two to four of the tubes – will contain 1.5ml of RNA later. The remaining two to four tubes will contain Allprotect™.

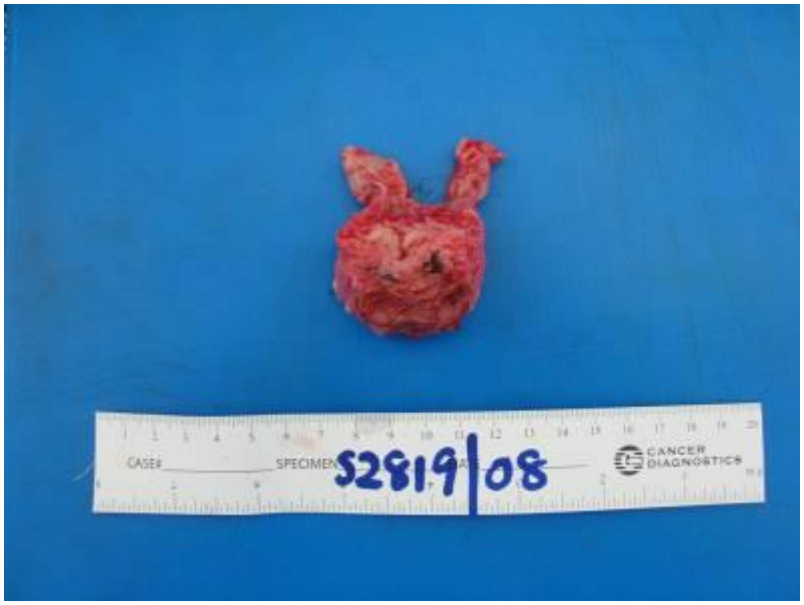
1.2.12 Label two to four cassettes in duplicate, for specimens to be snap frozen and fixed in 10% Buffered Formalin.

1.2.13 It is important to label the cryovials according to the specimen part being processed. Check the specimen card (i.e. Requisition Card) and label the vials accordingly (e.g. A1 & A2, B1 & B2, C1 & C2, etc). In some cases a frozen

section may have already been taken by hospital staff and labelled as A1 in which case the banked samples must be labelled from A2 onwards.

1.2.14 Fill the liquid nitrogen flask from the supply at the back of the cut-up room (always wear the face visor and gloves provided).

1.2.15 Once the Pathologist has weighed and measured the sample a photograph is taken, see below:




1.2.16 The Pathologist inks the Prostate as follows:

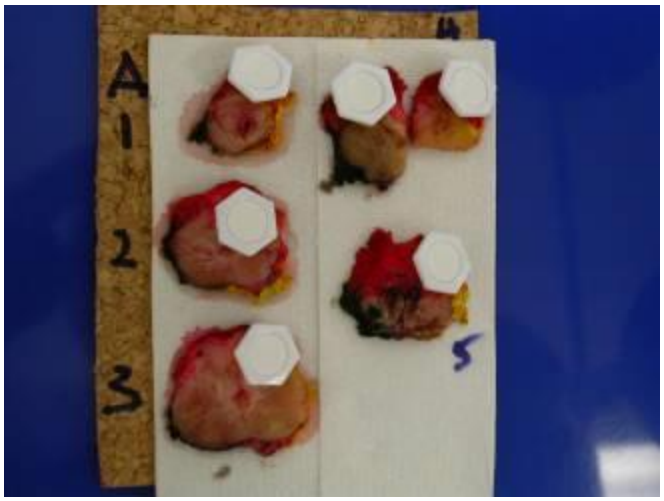
Anterior – Red
Right Lateral – Yellow
Left Lateral – Black

1.2.17 Following the inking of the specimen a photograph is taken, see below:


1.2.18 Once the pathologist cuts the Prostate into sections no more than 5 mm thick and places in chronological order a photograph is taken, see below:

1.2.19 The Pathologist cuts a wedge shaped section from a slice which will be processed for the Tissue Bank, see picture below:

1.2.20 The remaining tissue is pinned on tissue paper in slice order on a cork board and placed in a bucket containing formalin.




1.2.21 Depending on the size of the tissue wedge, cut into two pieces – A1/A2, B1/B1, C1/C1 (if unable to cut into two good size pieces, leave the wedge uncut) and lie the pieces on their side. Each piece is then cut into half, giving two A1/B1/C1's and two A2/B2/C2's. The outside of each half of each slice is then cut away, see picture below:


**Please Note:**

The above outlined method is the preferred method for dissection of the Prostate. However on occasion when Prof. Kay is unable to dissect the prostate, the pathologist on cut up may prefer to slice the prostate in half and Cut a wedge shaped section for Tissue Banking from one of the halves (As seen in the photograph below)

1.2.22 The small sections removed from the outside of each slice are cut into small pieces and divided between the vials containing Allprotect™ and RNA*later*®

1.2.23 The amount of tissue in the Alprotect™ /RNA*later*® samples must not take up more than a quarter of the volume of Allprotect™/RNA*later*®. Ensure the tissue is cut up into pieces no bigger than 2 x 2mm in size. This allows the Allprotect™/RNA*later*® to access and preserve the tissue much quicker resulting in higher quality samples.

1.2.24 One half of the remaining tissue slice is placed face down onto a piece of tin foil. The foil is folded around the tissue, placed into a cassette and immersed in liquid nitrogen.

1.2.25 The corresponding other half is placed face down on a piece of tissue in such a way that on embedding the first section taken corresponds to the first section taken from the snap frozen tissue. The tissue wrapped slice is then placed in a cassette and transferred to a beaker of 10% Buffered Formalin for fixing.

1.2.26 Ideally it should take no longer than 20 minutes to process the sample from when it is removed from the patient.

1.2.27 Record on the specimen requisition card the samples taken and record that is was taken for the Beaumont/RCSI tissue bank. For example:

Received fresh,
Samples taken for Beaumont/RCSI Tissue Bank from slice X:
A1 – A2

1.2.28 Photocopy the requisition card and leave the original at Beaumont Hospital specimen reception with the formalin sample.

1.2.29 On return to the RCSI Specimen Processing room place the RNA*later*® samples on the roller and leave for 24 hours. The samples in the container of 10% Buffered Formalin are placed on the roller for 24 hours before processing. Place the Allprotect™ samples in the 4 degree fridge for 24 hours.

1.2.30 At this point complete all details on the Tissue Banking – Prostate Details form (LF-HIS-TBProst).

## *1.3 Storage of Tissue Samples*

1.3.1 Place the snap frozen samples into the –80°C freezer.

1.3.2 The samples must be stored in the appropriate box. Place the samples in the box labelled TB Prostate Box and take note of the box number.

1.3.3 After 24 hours incubation in Allprotect™/RNA*later*® place the Allprotect™/RNA*later*® samples into the -20ºC freezer and record the time and date on the Tissue Banking – Prostate Details form.

1.3.4 The Tissue Banking – Prostate Details form (i.e. after completion) and the photocopied requisition form must be stapled together and filed in the allocated folder.

1.3.5 Enter all the details from the Tissue Banking – Prostate Details form and any other relevant information into FreezerworksUL3 (See CP-HIS-FWUL3 - Use and management of "FreezerworksUL3" for RCSI Tissue Banking).

1.3.6 Freezerworks can be found on the C/ drive or desktop of the first PC on the left in the RCSI Reading Room. Make sure that the position of the aliquots in each freezer correlates with that entered in Freezerworks.