Brisbane Breast Bank

SOP: Breast Tissue Collection and Processing

Breast Pathology Laboratory
University of Queensland Centre for Clinical Research

Tissue Collection

We collect tissue samples from patients that have consented to take part in the study. We collect both normal and tumour tissue samples. Theatre staff will contact the tissue bank staff when the specimens are ready for collection.

- Collect samples from the specimen room on level 4 of the Ned Hanlon Building.
- Transport the samples on ice to anatomical pathology located on level 2 of block 7.
- Photocopy the consent form and pathology request form and give the pathology staff the photocopy of the consent form and the original pathology form.
- Bring back the original consent form and photocopy of the pathology request form back to the breast pathology laboratory at UQCCR.
- The pathology staff will inform you if there are any tissue samples available for research after the pathologist has examined it.
- If the pathology staff inform you, that they have tissue samples for the tissue bank please go ASAP to collect the samples and transport them on ice back to the breast pathology laboratory at UQCCR.

Tissue Processing

Overview

Log the sample in the tissue bank log book and provide the sample with the next consecutive Tissue number (Q#) to de-identify the sample.

The following aliquots are required ideally:

Tissue	Aliquots
Normal Breast Tissue	4 X Frozen Normal Breast Tissue plus matching FFPE blocks
Breast Tumour Tissue	4 X Frozen Breast Tumour Tissue plus matching FFPE blocks

Note you may not get enough tissue for four aliquots and sometimes you may have tissue for more than four aliquots. There may be special requests for OCT blocks for particular samples as well.

Safety Procedures

Treat all tissue samples as potentially infections.

- Wear personal protective equipment (PPE) at all times: lab coat, safety glasses, double gloves.
- All tissue processing is to be performed in the biohazard hood in room 620.
- Use two sets of bench coat.
- Tissue waste is stored for a week in a 10% buffered-formalin container in the fridge (see cleanup procedures below for discarding tissue waste). **Ensure formalin is always opened in the fume hood as it should not be inhaled. It can also cause burns so always wear PPE.**
- All other non-liquid waste is to be double bagged, sealed and discarded in the biohazard bin.

Materials Required for Breast Tissue Processing

All materials required for processing can be found in the drawers near the biohazard hood. Place all items (tubes, racks, pipettes etc) to be used into the hood **BEFORE YOU BEGIN**.

List of items to be placed inside the hood (per patient):

- Sterile forceps
- Scalpel blade and handle
- Petri dish
- Nunc 1ml cryotubes
- Tube rack
- screw-top jar with 10% buffered formalin
- Embedding cassettes
- Biodyne bottle
- Double clear plastic bags (one inside the other)
- Two sets of bench coats

Items to place next to the hood:

- 70% ethanol and cavacide for cleaning the hood
- esky with ice
- paper towel
- gloves

Sectioning Breast Tissue for Freezing and Formalin Fixing

- Take the tissue to the Breast Pathology Laboratory's Biological Safety Cabinet in the primary tissue culture room. Spray the hood with 70-80% ethanol. Wear safety glasses, gloves and gown while processing the tissue.
- Label the NUNC 1mL cryovials with the appropriate Q#, type of tissue (normal or tumour) and date of collection. Normal tissue is labelled as NB (Normal Breast), Tumour tissue as BT (Breast Tumour). Each vial should be labelled as NB-A, NB-B or BT-A, BT-B etc. Also, label the embedding cassettes with the Q# (at the front), type of tissue (on the side) and date of collection (on the other side).
- In the hood under sterile conditions, using sterile forceps and scalpel-blade handle cut the tissue piece into approximately 4-5mm pieces and remove the fat (yellow) from the tissue if necessary. Then for each tissue piece: cut a piece out of the middle and place it in the embedding cassette. Then using forceps, place the two side pieces of the tissue into a NUNC 1mL cryotube. However, if the tissue is very small, then cut just a piece from the side and place into an embedding cassette, then place the other piece into the cryovial. Examples for various tissue pieces are shown in the diagrams below.
- Label both the embedding cassette and cryotube from the same piece of tissue with the same aliquot label e.g. both will be NB-A. This indicates the correct corresponding FFPE block for a particular frozen vial.
- Repeat all steps for each tissue piece aliquot.

*Aim for each vial to have a 6 mm maximum size of **tissue***

Size between 2-4 mm

Cut only a tiny piece of tissue from the edge for the cassette and place the rest in the cryovial.

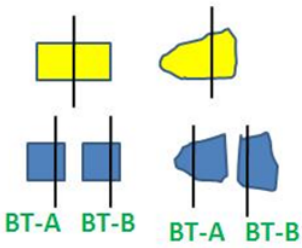
Size between 5-7 mm

Cut the tissue piece in two halves and cut a small representative from the middle for the cassette and place the corresponding tissue halves in the same cryovial.

Thin elongated piece: 5-7 x 3-4 mm

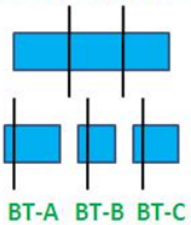
Orientate the fragment according to its long axis and cut the bottom lower part and place it in the cassette and then place the remaining tissue in the cryovial.

Tissue of 8-12 mm



If the tissue piece is too big for one vial (we want up to 6mm of tissue per vial), cut the piece in two halves and then cut a small representative piece from the edge. Place each small edge piece in separate cassettes and place the corresponding tissue pieces in separate cryovials.

Tissue from 1.2-2 cm or more



Make three vials each with their corresponding cassettes.

Snap Freezing Procedure

- Place tubes into esky of ice and take down the esky to the liquid nitrogen room on level 2 of UQCCR. Also, take with you: paper map sheet, pen and forceps. When in the liquid nitrogen room, put on green lab coat, face shield and blue cryogloves.
- Take out the particular tower from the liquid nitrogen tank and then place the tubes in the appropriate box. Then place the rack back in the liquid nitrogen tank.
- Record the position and the location of the samples in the paper map sheet.

Formalin Fixed Paraffin Embedded (FFPE) Block Procedure

- Place the embedding cassettes into the jar of 10% formalin. Leave the embedding cassettes in the formalin jar in the FRIDGE for 24 hours (unless it's Friday, in which case you would leave it in formalin until Monday). After 24 hours, using the fume hood and forceps, transfer the cassettes into a screw-top container with 70% ethanol. You can leave in 70% ethanol in the fridge for a week.
- Every Monday morning, take the formalin jar containing the cassettes with tissue over to the Histotechnology facility at QIMR. Put cassettes into the large 70% ethanol processing jar (usually in fume hood or fridge). Inform histology lab staff and let them know that it needs to be put on the 12 hour program. Ask histology staff for help if unsure of the protocol. Fill out their cost sheet (the UQ School of medicine sheet).
- Collect paraffin blocks from histotechnology facility when processed. Histotechnology phone number is 3845 3740.
- Each fortnight or each month, the paraffin blocks will be cut sections for H&E slides by Lynne, Jamie or Ashwini.
- Diagnosis will be made by the pathologist in our lab (currently Ekta is doing this) and recorded on internal diagnosis sheets.
- Store the blocks in the tissue bank cabinet. File the H&E slides in the tissue bank slide cabinet. Enter the internal diagnosis into the Access database and file the internal diagnosis sheets.

OCT Block Procedure

- OCT blocks are used for frozen sections.
- There may be special requests for OCT blocks for particular samples.
- In an aluminium foil mould, made using a 15ml tube cap squirt a small amount of OCT to cover the base, place the piece of tissue and then cover with OCT.
- Place the mould on top of dry ice (don't embed it into the dry ice) (never straight into -80^{0}C freezer) and allow it to solidify and turn white. This will take about 10 to 20 minutes.
- When solid, place the OCT block in a snap-lock bag and label appropriately and place in -80^{0}C freezer in the appropriate OCT box.
- Record location in excel sheet map and access database.

Cleanup

- Wipe the forceps with biodyne overnight, wash with cavacide and use brush to clean. Wipe the forceps with 70% ethanol. Place left-over tissue (e.g. fat) into 10% buffered-formalin container to fix the tissue and leave it to fix for one week at 4^{0}C. Place empty petri dish in double-lined plastic bag, tie bag with tape and discard into yellow biological waste bin.
- Wipe the biohazard hood with cavacide, rinse with water and then with 70% ethanol.
- Press down arrow to turn the shield down and then turn the hood off.
- Tissue waste is stored for a week in a 10% buffered-formalin container in the fridge and then the formalin is discarded in the formalin chemical waste container in the fume hood and the tissue is double bagged, sealed and discarded in the biohazard bin after the formalin has evaporated in the fume hood.