

TISSUE BANK TISSUE PROTOCOL

PROCESSING OF TISSUE

Treat all tissue as potentially infectious.

Tissue must be retained for at least 2 weeks before homogenising or cutting in the event that the pathologists may need the tissue for diagnostic purposes.

Tissue for clinical trials, which is consented specifically for a clinical trial, will not be seen by a pathologist and is available immediately, providing all ethics requirements have been met.

Tubes and cassettes must always have 2 forms of identification on them.

Label all tubes and cassettes with bar coded labels of specimen number and write on person ID number and whether the tissue is tumour or normal.

TISSUE COLLECTION

The intact operative specimen is sent as soon as possible to Anatomical Pathology fresh as for a frozen section. Some samples may be collected directly from the operating suite. Pathology must be notified.

Tissue bank staff will be present in Anatomical Pathology to freeze and fix the tissue as soon as possible to maximise the RNA preservation. The approximate time that elapses before freezing and fixation must be noted.

The pathologists will examine the specimen and, allowing for adequate tissue for histological diagnosis and assessment of margins, will remove a portion of the tumour and adjacent normal tissue for the Tissue Bank. Existing arrangements for storing tissue will take priority to the Tissue Bank collection.

For both tumour and normal, the tissue is dissected. Fixation may be in 70% alcohol (protocol C), 10% formalin (protocol B) and snap frozen (protocol A). Other types of fixation and storage arrangements may be made with the tissue bank. This is particularly the case for clinical trials

STORAGE

70% ethanol fixed Paraffin

10% formalin Paraffin

Unhomogenised tissue LN₂

Homogenised tissue aliquots LN₂

TISSUE INFORMATION RECORDED IN THE DAYBOOK

Specimen ID
Name
Date of Birth
Person ID
Hospital
Surgeon
Tissue type
Consent form
Date Blood Taken
Date Blood Processed
Amount of Blood

TISSUE BANK TISSUE PROTOCOL

Breakdown of blood components for storage
Blood comments
Time to freeze/fix tissue
Time blood collected, time serum and plasma frozen
Number of normal and tumour blocks fixed in 10% formalin
Date blocks processed
Number of normal and tumour pieces frozen
Data entered on database
Additional specimens taken
Storage of additional specimens
Any additional information/comments

A. SNAP FREEZING PROTOCOL

1. The tissue, approximately 4-5mm thickness, is placed into a plastic disposable histokinette cassette labelled with the specimen number, person ID number and whether the tissue is tumour or normal.
2. The cassette is wrapped in foil and a specimen number sticker is placed on the outside of the foil parcel. The parcel is then put into the liquid nitrogen flask.
3. On returning to Peter MacCallum Cancer Institute, the tubes are transferred to the -70°C freezer for the 2 week tissue embargo period.
4. The tissue, which is to be stored whole in as large a piece as possible, will be transferred to sealed Nunc tubes with a brown lid, numbered and catalogued, before being stored in liq N2 for long term storage.

B. 10% FORMALIN FIXATION PROTOCOL

Tissue, in plastic histokinette cassette as per collection protocol, is placed into 10% formalin for a minimum of 4 hours.
The blocks are processed by Peter Mac Histopathology staff according to standard protocols.

The blocks are embedded in paraffin wax.
A section is cut from each block and stained with Haematoxylin and Eosin.
Sections undergo a pathology review by a pathologist
Review data is recorded in Tissue Bank Database.

C. 70% ALCOHOL FIXATION PROTOCOL

1. Tissue is placed into 70% alcohol made with DEPC water for a minimum of 4 hours.
2. The tissue are transferred to a cassette labelled with the specimen number, person ID number and whether the tissue is tumour or normal.
3. The blocks are processed on the Tissue Tek VIP processor using program 9:

PROGRAM 9					
STATION	SOLUTION	TIME	TEMP.	PRESSURE	AGITATION

TISSUE BANK TISSUE PROTOCOL

				VACUUM	
3	70% alcohol	1:00		on	on
4	90% alcohol	1:00		on	on
5	100% alcohol	1:00		on	on
6	100% alcohol	1:30		on	on
7	clearant	1:30		on	on
8	100% alcohol	1:30		on	on
9	xylene	1:30		on	on
10	xylene	1:30		on	on
11	wax	1:30	60°C	on	on
12	wax	1:00	60°C	on	on
13	wax	1:00	60°C	on	on
14	wax	0:00		on	on

The program ends at 9am.

4. The blocks are embedded in paraffin wax.
5. A section is cut from each block and stained with Haematoxylin and Eosin.
6. The sections are imaged and stored on the database computer.