



STANDARD OPERATING PROCEDURE

Title: Fresh Tissue Collection, Processing and Storage

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Related SOPs

SOP 4.1.002 Donor Recruitment and Obtaining Informed Consent

SOP 5.1.006 Data Entry: Blood and Tissue Samples

SOP 5.1.012 Instructions for Label Printing for Cryovials

SOP 5.1.016 Data Entry: Management of Storage Containers

SOP 10.1.004 Procurement of Formalin Fixed Tissue

Revision History

Version	Issue Date	Reason for amendment	Sections amended	Replaces Version	Revised by (initials)
1.0	2006	Original document	n/a	n/a	JEC
1.2	22/12/2008	Review and reformat (version 1.1 skipped)	All	1.0	JEC
1.3	01/01/2009	Review and reformat	All	1.2	JEC
1.4	15/07/2016	New template and details added Change of title	All	1.3	JH



Purpose

Fresh frozen tumour and normal tissue samples are collected from surgical specimens when possible according to the judgement of a qualified pathologist. This SOP specifies guidelines to ensure that frozen tissue specimens from ABCTB donors are collected in compliance with ethical guidelines and processed in order to best meet the needs of research scientists.

Scope

Tissue collection from ABCTB donors is coordinated by Tumour Bank Officers (TBOs) with pathologists and medical staff at each ABCTB collection site; hence local variations to collection processes will occur. All tissue bank staff working within hospitals at collection sites must be trained and aware of local procedures. This SOP should be followed as an overarching guideline to best practice.

See also SOP 10.1.004 'Procurement of formalin fixed tissue' as this process is closely aligned.

Materials & Equipment

- Pre-barcoded cryovials

All frozen tissue specimens must be stored in the correct cryovials supplied with unique 10 digit barcodes beginning with the prefix for the site where they were processed. Wherever possible, vials should be used in barcode order. In preparation for tissue collection it is best to have a stored rack of empty vials in order ready for use. Use a sterile rack with a lid (e.g. a freezer box).

- Labels for aliquots and clear over-laminate labels
- Dewar of liquid nitrogen for snap-freezing
- Dry ice for transfer of specimens to storage

Method

Important: Treat all human biological material as potentially biohazardous and follow institutional safety procedures for handling and disposal accordingly.

1. Preparation and arrangements for specimen collection

- 1.1. Tissue samples collected for the ABCTB may only be retained once informed consent is obtained from the patient. It is best to consent donors prior to tissue sampling, however this may not always be possible at particular sites. It should be left up to the treating surgeon's and the pathologist's discretion as to whether a specimen may be suitable for tissue banking. If a sample is collected prior

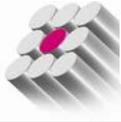


to consent, consent should be obtained as soon as possible after surgery. For any samples collected where consent is not obtained from the donor follow SOP 4.1.001.

- 1.2. Preparation for tissue processing should always be carried out in advance as much as possible to keep processing times at a minimum.
- 1.3. A tissue tracking form should be completed for each tissue collection from each donor. A copy of this form is attached as an appendix. Before the day of surgery, find out the surgeon, procedure and expected surgery date for each newly consented donor and fill out these details at the top of the tissue tracking form.
- 1.4. Prepare all of the materials and equipment listed above, including the printed labels for the vials. The table below shows the labelling format for normal and tumour tissue samples. Usually it will be possible to collect one or two fresh samples of tumour and normal tissue for each donor depending on the tumour. Always take a few extra tubes and labels than you expect to use in case more tissue is available. Refer to SOP 5.1.002 for label printing instructions.

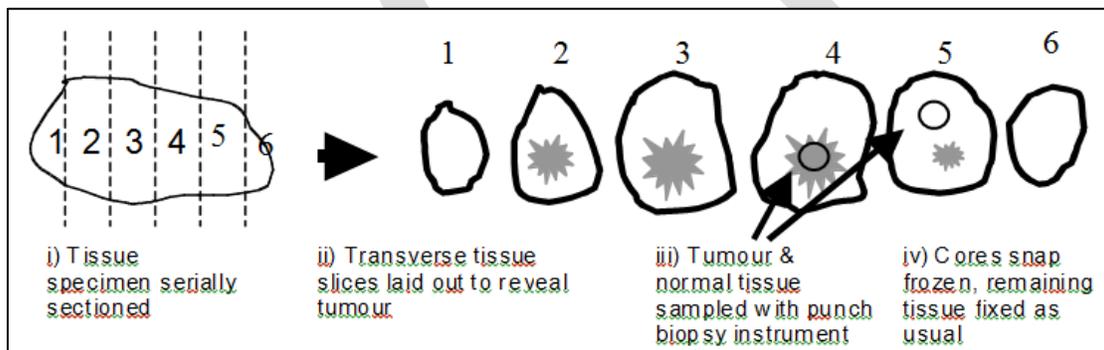
Sample type	Vial label	Cap insert colour
Tumour tissue	<i>TBRef#</i> 'Tumour' <i>dd-mm-yyyy</i>	White
Adjacent no-cancer	<i>TBRef#</i> 'Adjacent no-cancer' <i>dd-mm-yyyy</i>	Grey
Prophylactic/Normal	<i>TBRef#</i> 'Prophylactic/Normal' <i>dd-mm-yyyy</i>	Grey

- 1.5. On the expected day of surgery, check the theatre schedule to confirm that the donor's surgery will go ahead and to find out the theatre number and order of surgeries to approximate the time you will need to go to collect a tissue sample.
- 1.6. Coordinate with theatre staff and/or pathology staff so that you can be ready for sample collection at the histopathology lab when the donor's specimen arrives, and to prevent the specimen being directly transferred to formalin as per normal procedures at many labs.
- 1.7. It may work best to assist with transferring the specimen from the surgery. If you do this, be aware of log books you need to sign when checking specimens out of the surgery and into the histopathology lab. Always carefully check that specimens are labelled correctly and that you transfer specimens with the required pathology specimen request forms.
- 1.8. If your local arrangements involve entering the theatre, make sure you have the correct theatre attire (e.g. scrubs, cap, over boots and mask). Always cover your face with a mask when entering the theatre, and follow all other local protocols regarding theatre procedures.



2. Sample collection in the histopathology lab

- 2.1. When the surgical specimen/s from a consented donor arrives in the histopathology lab, note the 'Time specimen received in lab' on the tracking form and inform the pathology reception staff so that the specimen is logged into the laboratory computer system as per usual procedures.
- 2.2. Alert the pathologist who will be sampling the tissue that it has arrived.
- 2.3. If it is the local procedure for fresh sectioning, place the specimen at -20°C for the time specified to ease cutting, otherwise wait with the specimen on a cut up bench.
- 2.4. When the pathologist is ready to review the case he/she will decide whether it is suitable for tissue banking. Sometimes it will not be suitable, for example if the tumour is too small or too close to the margins, the pathologist may decide that fresh sampling is a risk to the donor's diagnosis. When this occurs, skip to step 2.8.
- 2.5. Wait while the pathologist documents the macroscopic features of the specimen and marks the excision margins as appropriate.
- 2.6. The method of sampling the tissue will be decided by the pathologist and may be either with a punch biopsy or directly with a scalpel. See the figure below demonstrating how samples may be taken using a punch biopsy on a serially sliced specimen.



- 2.7. If it is possible for the pathologist to take a sample of tumour and/or adjacent normal tissue for the tissue bank, open a pre-barcoded cryovial to collect each sample as soon as the pathologist is ready.
- 2.8. Label each cryovial and insert the appropriate cap insert as per step 1.3 above. When you label the vials, take care not to obscure the bar code and cover the printed labels with a clear over-laminate label. If possible, do this in advance while you are waiting for the samples.
- 2.9. Record the vial number, tissue type and approximate size of all samples collected on the tracking form.
- 2.10. Snap freeze the samples by submersion in liquid nitrogen and fill out the 'Time frozen' on the tracking form. Note: Depending on local protocols, frozen samples may need to be stored temporarily within the diagnostic laboratory until the pathology report is complete and the samples are released.



- 2.11. For cases where the donor had more than one tumour, it will usually only be possible to get a sample from the biggest one. Where possible make notes on the tracking form of any macroscopic description you have to identify the tumour/s you are able to get samples from (e.g. 'larger tumour', 'smaller tumour', '5cm from nipple', 'closer to margin'). This will help to match the pathology with the tumour bank samples when the report becomes available.
- 2.12. If the specimen was not suitable for collecting a fresh tissue sample, make a note of this on the tracking form and cross through the empty lines where the vials would have been recorded. If known, also note the reason why the case was not suitable.
- 2.13. Whether or not a case was suitable for fresh tissue banking, it may be possible to obtain formalin fixed tissue for the ABCTB after the specimen has been fully processed by the diagnostic lab. Details of formalin fixed tissue procurement should be filled out on the reverse side of the tracking form as seen attached here; go to SOP 10.1.004 to follow on with this procedure.

3. Specimen storage, data entry and filing

Note: Frozen tissue samples are stored in a liquid nitrogen vapour phase tank. As a precaution in case of equipment failure, where possible frozen specimens from each donor should be split into different storage locations. See SOP 5.1.016 for storage box naming formats.

- 3.1. Check the database for the next storage location for the frozen tissue samples and note the boxes and positions on the tracking form for each vial.
- 3.2. Transfer all of the frozen tissue samples from liquid nitrogen to their allocated locations. Take a box of dry ice to keep specimens and vapour tank boxes as cold as possible whilst you are moving the specimens into their storage locations.
- 3.3. Enter all of the details recorded on the tissue tracking form into the database following SOP 5.1.006. Sign the bottom of the tracking form when complete.
- 3.4. File the tracking form away with the donor's other paperwork.

Safety

Institutional safety requirements must be adhered to at all times.

Personal protective equipment such as gowns and gloves must be worn at all times when in the laboratory.

All human sample material must be handled in a Class II Biohazard Cabinet.

All local Chemical and Sharps policies must be adhered to.



Ordering Information

Supplies for frozen tissue collection and storage boxes are ordered and stored at the Central Processing Laboratory at Westmead. Contact the TBO at Westmead when stocks are getting low to allow time for ordering if necessary.

Item	Manufacturer	Supplier	Product name	Catalogue number
Pre-barcoded vials	Greiner	Interpath Services	Greiner per bar coded cryovials	122263-128C
Labels	GA-International	Molecular Solutions	CryoLabel, White, 25.4 x 12.7mm	TTCL-7
Clear label cover	GA-International	Molecular Solutions	Vial Clear-Wraps 51 x 25mm	CF-1
Freezer boxes	Greiner	Interpath Services	Greiner Cryobox Pc - 2MI 10X10 - Assorted	575019S

Stick addressograph sticker here OR

Patient's name:

Patients MRN:

Hospital:

Tissue Tracking Form

Tissue Bank Ref# _____	Surgeon: _____
Surgery date: _____	Procedure: _____
Surgery time: _____	Expected material type (circle): Fresh / FFPE

Fresh specimens –

Collection date: _____ Histopathology lab: _____

Collection time: _____ Pathologist: _____

Time received in lab: _____ Pathology reference # _____

Vial No.	Tissue Type (Tumour, Adjacent normal etc.)	Size (mm)	Storage Box	Position	Freezer/Shelf/Rack

For adjacent normal tissue: distance from tumour (mm): _____

Date frozen: _____

Time frozen: _____

Notes:

Entered in database by: _____

Tissue Tracking Form

Formalin fixed paraffin embedded (FFPE) blocks –

Block path reference #	Block type (circle)	Block suffix (E.g. '.1A')	Tissue Type (Tumour, Adjacent normal etc.)	Storage Box (E.g. WMD Blocks 2011)	H&E slide in bank? (Y/N)
	.EX / .LB				
	.EX / .LB				
	.EX / .LB				
	.EX / .LB				
	.EX / .LB				
	.EX / .LB				
	.EX / .LB				
	.EX / .LB				

Block types:

'EX' = Exclusive block assigned to the ABCTB. Exclusive blocks should be stored in the tissue bank with an H&E slide where possible. You do not need to record slides from exclusive blocks as separate specimens.

'LB' = Lab block which remains the property of the pathology laboratory. Only an H&E slide will be stored with the tissue bank for these cases.

If 10% neutral buffered formalin (NBF) was not used, please state the name of fixative: _____

Notes:

Entered in database by: _____