



CCLG Tissue Bank Guidelines

Guidelines for the storage and registration of paediatric tissue specimens for research

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Tissue Banking – Laboratory Guidelines

The importance of biological studies into paediatric cancers requires access to a large series of samples of children's tumours. These samples need to be of high quality, be pathologically verified and linked to relevant clinical data.

In order to support the tissue banking of children's cancers for the CCLG Tissue Bank, the following laboratory guidelines have been proposed. These guidelines are to help biomedical scientists (BMS), nurses and pathologists with any technical queries, and to offer suggestions for best methods of practice to adopt within the laboratory.

Each local CCLG centre will already work to standard operating procedures within their own laboratories, however further clarification is required for the collection, registration and release of samples for the CCLG Tissue Bank and biological studies, which is vital in supporting paediatric oncology research.

Sample Selection

Histopathology and neuropathology samples of frozen tissue and paraffin embedded tissue can be registered with the CCLG Tissue Bank.

The types of specimen that can be stored in the bank include:

- Frozen solid tumours
- Normal tissue adjacent to solid tumours
- Positive bone marrow samples at diagnosis
- Frozen whole blood
- Extracted DNA, RNA, and protein from whole blood
- Formalin fixed paraffin wax embedded tumour and normal tissue samples
- Cells or tissue sections on microscope slides

To ensure maximum potential of paediatric samples that can be banked, it is advised to always freeze at least one aliquot of tissue from all paediatric cases received in the laboratory. This can only be permitted following consent and when there is plenty of tissue in surplus for diagnostic requirements. However, if laboratories ensure that tissue will subsequently be available for potential banking, through sampling in the first place, the resource can always be accessed if consent is confirmed later.

Sample Storage

Frozen tissue

There are various approaches to how samples are frozen and stored within histopathology and neuropathology laboratories.

The majority of laboratories freeze and store tissue at -80°C . However storage in liquid nitrogen is the preferred method to best maintain tissue integrity. Initially snap-freezing tissue in cryovials in liquid nitrogen, and then transferring to -80°C freezers is the best option and the most desirable method for handling and storing tissue. The type of sample storage methods used within centres is dependent upon the facilities available. To facilitate future transport of samples it is advisable that all samples be stored in small cryovials (e.g. 2 ml capacity) with samples of 1 aliquot amounting to at least $0.5\text{-}1.0\text{ cm}^3$ amount of tissue, where possible.

Specifically, rhabdomyosarcoma specimens need to be rapidly frozen once collected in order to preserve the molecular integrity of the tissue. The best practice for these samples should be to place in vials onto dry ice or in liquid nitrogen. Where this is not possible, samples should try to be kept cold by storing on ice or in a fridge prior to freezing.



Where there is only a frozen section prepared block of tissue available from a patient, this can still be registered with the tissue bank. OCT embedded tissue is not ideal for banking, however it is better to bank these tissues than not at all.

Whether or not the specimen is stored separately from other surgical biopsies is the responsibility of each department. Large centres which collect numerous specimens may benefit from storing registered CCLG tissue specimens separately. However, this may not be justified for centres which only bank small numbers of paediatric specimens. Some centres may have their own local tissue bank, where these samples can be transferred from the lab to this tissue store. Therefore the storage of sample is the sole responsibility of the centres. It does not matter where the locations of the samples are, but it is important that it can always be determined by laboratory staff. It is vitally important all laboratories keep an up-to-date log of specimens and related information, of both electronic and hardcopies where possible, as this will facilitate storage and retrieval of specimens.



Important consideration must be given to the level of detail transcribed onto stored tissue cryovials, to identify specimens. Patient anonymity must be observed when releasing specimens, in which case tubes must only have the designated Centre Number and CCLG case number on them when they leave a centre for despatch to a researcher (see section – *Specimen Release*)

Paraffin blocks

If the pathologist wishes to register a paraffin block with the tumour bank, they must ensure that the tissue has been assessed as part of the diagnostic process. This ensures that if the block is requested for release by an approved CCLG biological studies researcher, the pathology of the tissue has previously been determined. This tissue must always be stored within the department as part of a patient's diagnostic record, therefore researchers should readily return this resource in good condition after it has been used for the requirements of the study.

Constitutional DNA (from whole blood)

Constitutional DNA is centrally stored for the CCLG Tissue Bank. To bank constitutional DNA, a vial of fresh blood must be sent to Leeds (to Andy Sharpe) in the provided inner containers which are placed into transport specimen boxes and posted together with a copy of the completed DNA registration Form B. A copy of Form B must also be sent to the Tissue Bank Manager at the CCLG Coordinating Centre, Leicester.

Registration of Tissue Samples

The person designated to initiate the completion of the specimen registration forms is usually the centre's data manager, research nurse/clinical trial co-ordinator or BMS (if you are not sure who this is then contact the CCLG Coordinating Centre prior to registering specimens). This person is responsible for confirming that a patient has given consent for their surgical biopsy or resection to themselves or a clinician, to be registered and used by the CCLG tissue bank. They will allocate a CCLG case number* to each patient. The same case number is used for all samples of a patient, unless they develop a second malignancy in which case a new case number is allocated. It is the responsibility of the pathologist to assess the availability of paraffin and frozen tissue for banking, providing details of the specimen and storage location.

Specimen Registration – Form A

Form A must be completed for all samples registered except for constitutional DNA. The details required on Form A allows an informed registration of a sample with the CCLG Tissue Bank, and ensures that appropriate information is available about these samples upon release to CCLG approved biological studies. Please use a separate form to register each sample.

Information required on Form A includes:

1. Centre Number (see CCLG tissue bank protocol for list of centre identifier numbers).
2. CCLG Case Number – allocated for each new patient by a designated person at each centre
3. Patient Identifiers – full name; date of birth; gender; hospital number
4. Confirmation of consent details (usually completed by research nurse/data manager)
5. Specimen Information – (*N.B Each type of specimen has to have a separate form A completed*)
 - Date specimen taken
 - Laboratory specimen number
 - Pre/Mid or Post chemotherapy and Pre/Post radiotherapy (when applicable)
 - Specimen type – biopsy/ resection/ bone marrow/ blood/ other
 - Normal/metastatic and primary/secondary tumour
 - Site of sample
 - Diagnosis (including WHO grade where applicable) – stage; date of diagnosis

- Specimen storage – type of specimen stored; number of aliquots of frozen tissue or paraffin blocks; storage location details (shelf, rack and freezer numbers)

Please ensure the form is signed to confirm that samples have been stored appropriately and will be made available for CCLG biological studies. If the sample is lost, damaged or used for local research please inform the CCLG Co-ordinating Centre, so we can update our records.

Constitutional DNA Registration - Form B

The details required to complete Form B are the same as that on Form A (as described above). No information on storage is required, as these samples are centrally stored for the CCLG Tissue Bank (DNA labs, Leeds). Details on the form must be provided in full for patient names and diagnosis. DNA that is stored locally in centres which is available for research can still be registered with the tissue bank, in which case the storage details must be stated on this form.

Personalised specimen and DNA registration forms for each centre can be found on the 'Biological Studies Steering Group' of the CCLG Website: www.cclg.org.uk

All completed Forms A and B with a copy of the **pathology report** attached must be sent to:
 Gita Mistry, Tissue Bank Manager,
 CCLG Coordinating Centre, University of Leicester
 3rd Floor Hearts of Oak House,
 9 Princess Road West,
 Leicester. LE1 6TH

Sample Release

If a researcher with an approved biological study wishes to use tissue from the CCLG Tissue Bank, a formal request is made via the CCLG Coordinating Centre. It is the responsibility of the researcher to facilitate and pay for the transport of specimens from the local centre laboratories. Form 1 will be sent with the specimen release request from CCLG. This will have details of both the CCLG Centre and Case No. and pathology reference number; however the researcher will only be aware of the CCLG case No. to maintain patient anonymity. Both the researcher and Tissue bank manager (as above) are sent copies of completed Form 1 upon despatch of specimens by the centre, ensuring the pathology reference numbers are detached when copying to the researcher. The form is then completed by the researcher upon receipt of specimens and a copy is sent to the Tissue bank manager. Therefore Form 1 confirms the despatch and receipt of tissue for biological studies. The availability of tissue is then updated on the CCLG tissue bank database.

Form 2 will be sent for the constitutional DNA release request from CCLG. This will have details of the CCLG Centre No., Case No. and lab reference No. The form should be completed and signed by the DNA labs in Leeds releasing DNA samples and by the researcher upon receipt of DNA samples, and copies sent back to the Tissue bank manager.

Tissue Banking Payments

Payments per sample are: Paraffin Block = £15.00
Frozen Tissue = £125.00

This is for each patient per surgery time point, regardless of multiple blocks or aliquots of tissue that are registered (i.e. if 2 aliquots of frozen tissue are banked from 1 patient, the centre will still be paid £125.00, and not £250.00).

** The CCLG tissue bank number consists of a single 3 part code for each sample registered. This code is the centre number, the unique patient case number, and a specimen code (which consists of a specimen letter and number). This makes it much easier to match up samples within the bank for each patient, identifying any biopsies, resections or relapses registered.*

Centre Number – centre at which the patient has been registered.

Case Number – unique patient identifier allocated by the data manager, research nurse clinical trials co-ordinator or BMS. Patients have the same case number for all of their samples.

Specimen code – a letter to indicate the type of sample.

- *F = Frozen*
- *P = FFPE (paraffin)*
- *D = constitutional DNA (sent to Leeds for central storage or locally stored)*
- *B = Blood (stored locally)*
- *M = Bone Marrow*

*- a number to indicate the next number for this type of sample (i.e. a patients first frozen sample will be **F1**, the next will be **F2** and so on).*

Centralisation

CCLG are currently proposing a centralisation of the CCLG tissue bank, so there will be a transition from the current virtual tissue bank where local centres are responsible for specimen storage and release to a centralised tissue bank.

In essence, laboratories will still be required to register and store specimens as they do now, however periodically all banked samples will be transferred to the central bank for storage. Researchers will then be able to request samples for release for their approved CCLG biological study without the need to contact local banking centres.

Any changes to practice will be notified to centres in good time, and any feedback as to how laboratories can deal with the new proposal of centralisation will be gratefully received.