

Quality of Life and Management of Living Resources

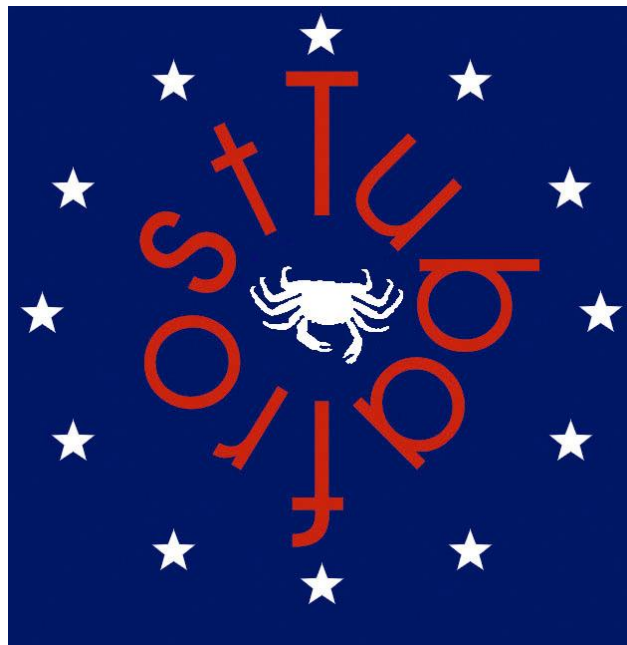
European Human Frozen Tumor Tissue Bank

TUBAFROST

QLRI-CT-2002-01551

Deliverable D 3.1

Protocols for collection and storage of human tumor and
corresponding normal tissue



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**TUBAFROST Work Package 3:
Protocols and systems for collection
and storage of tissue (Deliverable 3.1
June 2003)**

Standard Operating Procedure 1: Protocol for collection of human tumour and corresponding normal tissue

Introduction and Purpose

This Standard Operating Procedure defines the collection procedure of human tumour and corresponding normal tissue for the TUBAFROST project. The components of the collection procedure are the organisational structure, the specified time limit and the freezing technique.

Background Information

Reliable and reproducible results from the tissue banks of all TUBAFROST participants can only be accomplished by standardisation of the methods of tissue collection and freezing by the use of identical protocols.

Safety

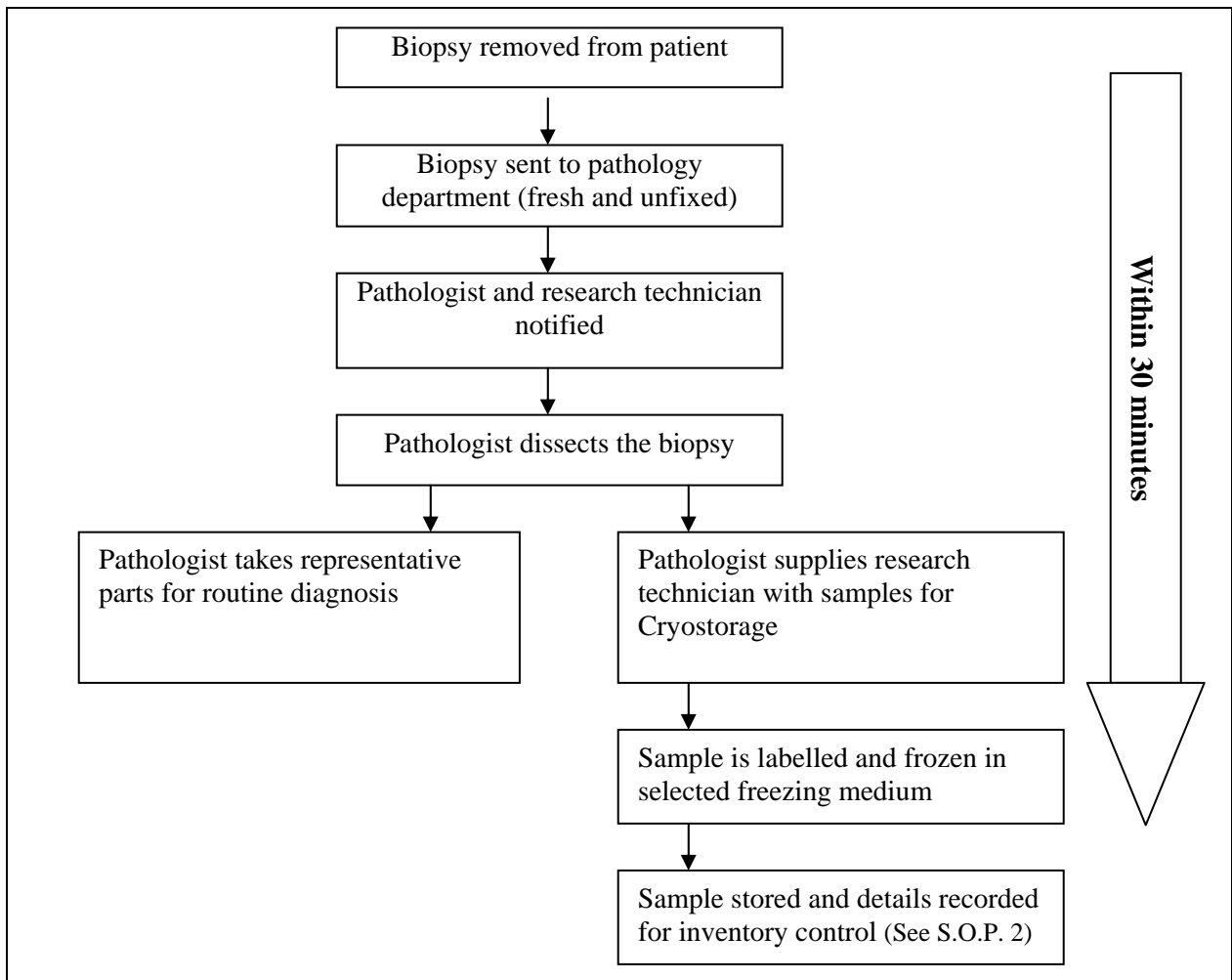
Carry out all procedures in accordance with the local codes of practice. Working with liquid nitrogen and isopentane is hazardous therefore all procedures should comply with local safety rules specific to these chemicals. All tissue must be treated as potentially infectious.

Associated Standard Operating Procedures

S.O.P. 2 Protocol for storage of human tumour and corresponding normal tissue

Procedure

There should be an organisational structure for the efficient collection and storage of tissue



- 1.1 Biopsy removed from patient in operating theatre
- 1.2 Biopsy immediately sent to pathology department fresh and unfixed (ideally in sealed sterile container)
- 1.3 Pathologist and dedicated Tubafrost research technician notified via pager. Immediate notification is necessary to minimise hypoxic phenomena. The Tubafrost project specifies 30 minutes as the maximum time from excision of tissue to snap freezing. The sample must not be allowed to dry out.

Pathologist

- 1.4 Dissect the biopsy using aseptic technique (new scalpel and clean instruments for each resection and cleaned/changed between dissecting normal and tumour tissue).
- 1.5 Take representative parts for routine diagnosis as priority and decide if there is sufficient material available for Cryostorage.

- 1.6 Supply research technician with samples for Cryostorage. These samples are ideally representative parts of the lesion, normal tissue and pre-malignant conditions.

Research Technician

- 1.7 Prepare sample for snap freezing using aseptic technique (clean surface and instruments, change instruments in between preparing normal and tumour tissue). The ideal size of tissue for snap freezing is approximately 1.0x0.5x0.5cms though the amount of tissue will differ depending upon the sample site. Smaller fragments can be snap frozen. If there is sufficient material freeze duplicate samples (therefore there may be many samples per biopsy).
- 1.8 Prepare the freezing medium by suspending a vessel of isopentane (2-methyl butane) in liquid nitrogen; this will bring the isopentane towards its freezing point (-160°C). The appropriate freezing point for the tissue approximately corresponds to the moment when opaque drops begin to appear in the isopentane. Tubafrost does not recommend the use of liquid nitrogen as the freezing medium, nor slow freezing in a -80 °C freezer.
- 1.9 Either embed the tissue samples in O.C.T. (optimal cutting temperature) compound and freeze in isopentane or freeze directly in isopentane. Do not remove the tissue from the isopentane until freezing is complete (10 seconds or less depending on size) but ensure sample does not crack. Remove sample from isopentane and enclose in a cryovial or other storage vessel.
- 1.10 Label the storage vessel with the TUBAFROST code (consisting of TF_institution number_sequential code) using waterproof permanent pen able to withstand long-term storage at low temperatures. The sequential code is the local inventory code and hence will not in any way relate to the pathology number or other identifiers. The Tubafrost recommendation is that bar codes should be used but the TUBAFROST code must also be included to make them human readable at institutes where there are no bar-code readers.
- 1.11 Follow Standard Operating Procedure 2 for the storage protocol.

References

Centro Nacional de Investigaciones Oncologicas www.cnio.es

European Union Lung Cancer Partnership www.euelc.com

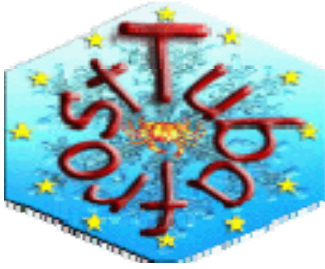
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National Cancer Institute Cooperative Human Tissue Network (CHTN) www.chtn.ims.nci.nih.gov

NCI Cancer Centre funded University of Florida Molecular Tissue Bank www.pathology.ufl.edu

Peterborough Hospital Human Research Tissue Bank www.tissuebank.co.uk

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Standard Operating Procedure 2: Protocol for storage of human tumour and corresponding normal tissue

Introduction and Purpose

This Standard Operating Procedure defines the storage procedure of human tumour and corresponding normal tissue for the TUBAFROST project. The storage procedure incorporates the specified storage mechanism, the alarm network, the back-up measures and the associated inventory system.

Background Information

Reliable and reproducible results from the tissue banks of all TUBAFROST participants can only be accomplished by standardisation of the methods of storage by the use of an identical protocol. Use in conjunction with Standard Operating Procedure 1.

Safety

Carry out all procedures in accordance with the local codes of practice. If a liquid nitrogen freezer is used particular attention must be paid to the local safety practices of working with liquid nitrogen. All tissue must be treated as potentially infectious.

Associated Standard Operating Procedures

Use in conjunction with S.O.P. 1 Protocol for collection of human tumour and corresponding normal tissue

Procedure

- 1.1 Transfer the snap frozen sample from the isopentane to a pre-chilled storage container for transfer to the chosen storage repository. The storage repository can range from a -80°C freezer to a liquid nitrogen storage facility in liquid or vapour phase. Tubafrost advocates the use of a liquid nitrogen repository. The actual storage system within the repository is unique to the institute but location co-ordinates must always be recorded.

- 1.2 The storage repository must have an alarm network in place. The Tubafrost project proposes a tri-phase alarm system with a) local visual and acoustic alarms where the storage repository is located, b) a distant acoustic and visual alarm in a central surveillance facility. If there is no central surveillance facility or in the event of neither local nor distant alarms attracting attention there should be c) a remote alarm capable of automatically dialling out pre-programmed telephone numbers.
- 1.3 There must be a back-up system for the storage repository; the Tubafrost minimum recommendation is for a back-up freezer running constantly. The Tubafrost ideal would be to store 2 identical samples independently, i.e. separate storage facilities.
- 1.4 Record details in the relevant inventory book. The Tubafrost standard method will be to have double storage of information, firstly in the inventory book and then in the computerised system. At a minimum the information recorded will include inventory number, location co-ordinates, pathology number, type of tissue and date.
- 1.5 Transfer details to the computerised database system, Tubafrost recommends that all participants use an electronic database for storing inventory information; this database should be linked to provide minimum datasets.
- 1.6 It is essential to update the database when samples are moved or depleted.

References

- Centro Nacional de Investigaciones Oncologicas www.cnio.es
- Helsing KJ, Hoffman SC, Comstocka GW. Temperature variations in chest-type mechanical freezers. *Clin Chem* 2000;**46**:1861
- Peterborough Hospital Human Research Tissue Bank www.tissuebank.co.uk
- Naber SP. Continuing role of a frozen-tissue bank in molecular pathology. *Diagn Mol Pathol*. 1996 Dec;**5**(4):253-9.
- Su SC, Garbers S, Rieper TD, Toniolo P. Temperature variation in upright mechanical freezers. *Cancer Epidemiol Biomarkers Prev* 1996;**5**:139-140
- The BOC group (hazard data sheets) www.boc.com



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Quality Control Draft Proposal for TUBAFROST Network

Quality control (QC) is only a part of the Quality assurance (QA) or quality policy, which is the final goal.

Quality Assurance includes:

- Written standard operating procedures
- Quality indicators
- Objectively Quality goals

Ideally a certified Quality Programme would be applied in each Hospital Tumour Bank (this is almost impossible) and/or in the Central Office of the network, especially when collecting and distributing tissue from many different institutes.

Quality Control is a part of the quality indicators.

A proposal:

➤ In each Hospital:

- Review of 2% of the new cases, twice a year.
 - Cases are selected at random but only from the common cases (colon, breast, lymph nodes, lung, appendix and tonsil). Don't use infrequent cases (brain, tumoural skin)
 - 2% review twice a year in the first year a hospital belongs to the Network. If no quality problems emerge then this can be reduced to 1% or similar
- Records and files:
 - Appropriate informed consent
 - Specimen receipt and patient identification correctly recorded (random check)
 - Clinical information: if necessary to establish the minimum datasets or the minimum data points which an acceptable file must contain: tumour stage, grade, size, localization sex, age.
 - Appropriate SNOMED codification
- Equipment
 - Technical reviews (protocols)
 - General maintenance of freezers, alarms and back-up systems

- Fixed tissues
 - Review of stained HE sections by a pathologist in order to assess/confirm the diagnosis and representativity of the sample.
 - Review of the sample identification (bar code, etc)
 - Test of fixation: immunohistochemical staining to evaluate optimal sample fixation (antigen preservation) in paraffin blocks. Vimentine, Ki67, CD34. Can use a tissue-array (1,5 mm in maximum diameter) that allows testing of antibodies in the same conditions, with a limited number of slides. This tool maintains the integrity of the original paraffin blocks. It would minimise the final cost.

- Frozen tissues
 - Reviews of stained HE sections by a pathologist in order to assess/ confirm the diagnosis and how representative it is of the sample.
 - Review of the sample identification (bar code, etc)
 - RNA extraction and quality assessment in an agarose gel or bio analyzer.

- In the Central Office:
 - About records and files:
 - Appropriate SNOMED codification (if centralised)
 - Registries of activity