

Quality of Life and Management of Living Resources

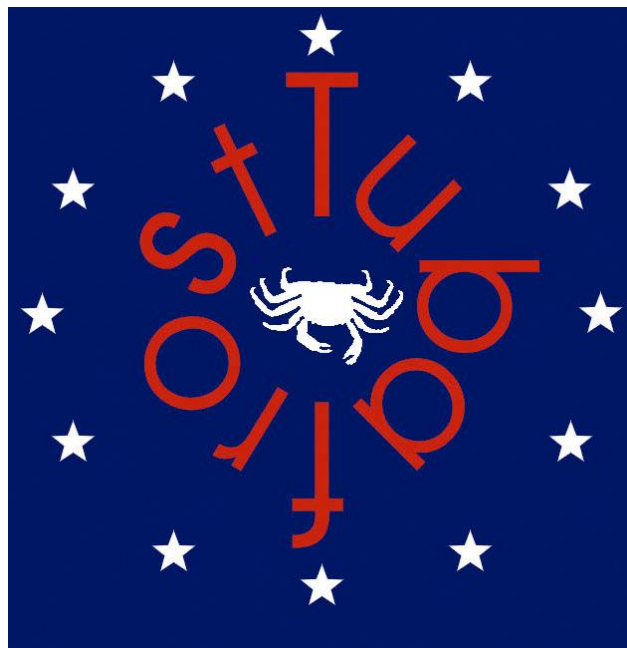
European Human Frozen Tumor Tissue Bank

TUBAFROST

QLRI-CT-2002-01551

Milestone 3.1

**A report on implementation of standard operating
procedures for the European Human Frozen Tumour
Tissue Bank (TuBaFrost)**



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Milestone 3.1 - A report on implementation of standard operating procedures for the European Human Frozen Tumour Tissue Bank (TuBaFrost)

CONTEXT

1. In 2002, European Framework V funding was awarded for the development of a virtual, networked European human frozen tumour tissue bank – the TuBaFrost project. Established as an initiative of the Erasmus MC Department of Pathology the TuBaFrost project is being developed in close collaboration with the European Organisation for Research and Treatment of Cancer (EORTC) and 10 other participants from seven European countries.
2. The objective of the TuBaFrost project is to create a resource of high quality frozen tumour tissue samples with corresponding accurate diagnosis stored in major European cancer centres and universities. The bank will be searchable through a query system on the Internet, which is provided with rules for access and use of the tissues complete with a European code of conduct to comply with the various legal and ethical regulations in European countries.
3. Further information about the TuBaFrost project and its participants can be found at: www.tubafrost.org. Each participant within the TuBaFrost project is responsible for taking forward or contributing specifically to one of the following work packages:
 - Program management
 - Development of storage system(s) for frozen tissue (technical aspects)
 - Protocols and systems for collection of tissue (logistics)
 - Hard and software for co-ordinated tissue storage
 - Virtual microscopy in tissue banks
 - Rules for use of stored tissue
 - Legal and ethical aspects of tissue banking
 - Educational aspects
 - The European Human Frozen Tumour Tissue Bank – evaluation.

The storage of tissue will be de-centralized, at the institute where it is collected. To assure equal quality of tissue, which in outcome of scientific experiments can be compared, standardization of the collection and storage methods is fundamental. Therefore, protocols for storage, retrieval and tracking of tissues will be standardised and implemented in all participating laboratories.

Centralised access will be provided to the de-centrally stored material via a central database containing representative histological images and coded-linked patient data. The proposed addition of images makes the tumour bank a "virtual tumour bank" and has the advantage that the diagnosis can be verified. The use of virtual microscopy to document the histopathological diagnosis will be investigated and implemented if the system appears feasible. Therefore, virtual microscopy must be made suitable for high throughput of microscopic slides and have sufficient storage capacity for large numbers of high quality images. The images will be stored in a database that can be linked to the coded clinical information and the location of the samples.

The database will be published in the restricted public domain, but must be freely accessible for the European scientific community via the web. The website (www.tubafrost.org) has been developed to publish and explain the use of the virtual tumour bank. To enable working with the tissue and thereby crossing European borders, a code of conduct will be developed by expert health-jurists for research with "left over" human tissues that complies with the various legal and ethical regulations in Europe. Incentives for collecting frozen tissue samples, access rules and use of the tissues will be established in dialogue with the participating institutes to ensure smooth operation of the bank.

BACKGROUND

Developing an operational framework for the TuBaFrost project

4. It is **recommended** that TuBaFrost operational policies and procedures:

- take account of existing International guidance, policies, procedures, and best practice worldwide;
- are formally validated by members of the TuBaFrost consortium through Deliverable 3.3 and Milestone 3.1;
- are embedded in practice at TuBaFrost participating centres;
- are assured through quality control policies and procedures; and
- are regularly reviewed by members of the TuBaFrost Steering Committee (Milestone 6.1).

In support of this, it is **recommended** that TuBaFrost operational policies and procedures include:

- TuBaFrost work flows and operating procedures (SOPs) to be employed at the collection centres for:
 - collection, handling and storage of frozen tissue samples;
 - coding of samples; and
 - obtaining complete inventory datasets.
- a quality control policy to ensure optimal standards of:
 - frozen tissue quality;
 - associated inventory records;
 - sample location mapping; and
 - tissue banking equipment.

Standard operational procedures for TuBaFrost will ensure:

- standardised high-quality of tissue, which in outcome of scientific experiments can be compared; and
- guidance for new collecting institutes to enable them to meet the demands set by the TuBaFrost consortium on minimum standards, protocols and quality control.

5. The development of a draft operational framework for TuBaFrost has drawn upon the best practice standard workflows and operating procedures employed by members of the TuBaFrost project and key initiatives worldwide. Following consultation with key members of these initiatives to assess current operational frameworks through February to June 2003, draft SOPs were developed for the collection, storage and quality control of frozen tissue samples for Deliverable 3.1, these can be found at **Annex A**. The quality assurance procedures have been developed in collaboration with the Tumour Bank Network, Centro Nacional de Investigaciones Oncológicas (CNIO), Madrid.

6. A detailed appraisal of the basic design for the storage system investigated further the storage recommendations made within Deliverable 3.1 and has drawn upon manufacturers information, relevant literature and a visit to the CNIO Tumour Bank Network, Madrid in November 2003. This enabled recommendations to be made for the design of the local storage system - which are detailed in Deliverable 3.2 at **Annex B**.
7. In order to develop and validate the draft standardised operating procedures, the quality control polices and the recommendations made in Deliverable 3.1, 3.2 and discussed at the TuBaFrost annual meetings a questionnaire was circulated to all participants in June 2004. This included TuBaFrost participants not directly involved in tissue collection and storage as it is necessary to ensure that the draft SOPs complement ongoing work across the consortium, for example compatibility with the development of the hardware and software. Consortium member responses are in full in Deliverable 3.3 at **Annex C**.

PURPOSE OF THIS REPORT

8. The purpose of this report is to:
 - reflect key points of feedback about the draft TuBaFrost SOPs;
 - highlight experimental data to support recommendations made in Deliverable 3.1 and 3.2; and
 - set out refined TuBaFrost SOPs for use at participating institutes for:
 - SOP 1: The collection of human tumour and corresponding normal tissue;
 - SOP 2: The storage of human tumour and corresponding normal tissue; and
 - SOP 3: The quality control policy for collection and storage of tissue.

KEY POINTS IDENTIFIED FROM CONSULTATION ON TUBAFROST DRAFT SOPs

9. To ensure standardised high-quality of tissue, which in outcome of scientific experiments can be compared, standardization of the collection and storage methods is fundamental. Standard operating procedures for collection and storage of tissues for TuBaFrost have been implemented in institutions participating in the TuBaFrost project and the following key points have been highlighted:

□ ORGANISATIONAL STRUCTURE

General

10. Establishing an organised method of collecting remnant human tissues for research or education should minimise the likelihood that operative specimens are compromised diagnostically (Grizzle 1998) It should also be of benefit to the pathology department as tissue will be transferred from the operating theatre to the pathology department more efficiently and it could help to address the shortage of pathology time available for supporting tissue banking activity. There needs to be adequate support to the tissue banking activities from the surgical and pathology teams: accountability structures and regular team reports and updates will help on this. Integrating the tissue banking activities into the routine surgical and pathology activities is essential for the efficient acquisition of tissue. Within the H. Lee Moffitt Cancer Centre, Florida the surgeons and pathologists work together to streamline the standard operating procedures to minimize warm ischemia and permit tissue freezing within 20 minutes – this is recommended as the standard for human tissue preservation prior to cDNA or oligonucleotide microarray analysis (Huang et al, 2001). This will be further discussed in the next section.

Implementation

11. As demonstrated by the Valencia Institute of Oncology, clear communication lines are key to establishing an organised method of collecting remnant tissue. To ensure adequate support for the tissue banking activities members of the surgical and pathology departments met and the TuBaFrost project was explained - with emphasis on the need for preserving fresh frozen tissue of quality to undertake research studies - and then the SOPs were introduced.

Additional points

12. Tissue bank personnel training must focus upon safety issues as well as specific tissue bank activities. It would be advisable that suppliers and users of tissue in a tissue bank certify in writing that they will train themselves and their personnel in the potential biohazards that human tissues represent (Grizzle et al, 1998).

□ LAG TIME FROM EXCISION OF TISSUE TO FREEZING

General

13. Reducing the lag time from excision of tissue to freezing is linked to the establishment of an organisational structure - if there is sufficient support from the surgeon and pathologist then the snap freezing lag time can be reduced.
Snap freezing the tissue must be done as soon as possible after excision to ensure there is minimal degradation of the specimen and hence no limitation on the types of studies that can be conducted or any influence on the scientific usefulness of the data obtained from tissue analysis. According to Spruessel et al (2004) control of variables such as tissue ischemia time is mandatory to obtain reliable data in screening programs for molecular targets and diagnostic molecular patterns.

Examples of sample handling logistics in key tissue bank networks:

- the Cooperative Human Tissue Network in USA regularly meets requests from researchers requiring tissue frozen within 20 minutes though this limits the number of specimens available and so the CHTN does not work to this limit routinely (Qualman 2004); and
- the Wales Cancer Bank UK and Chernobyl Tissue Bank advocate delivery of tissue to the pathology department from the operating theatre within 10-15 minutes and snap freezing as soon as possible.

Implementation

14. The lag time recommended for the TuBaFrost project is 30 minutes from excision to snap freezing, it is however recognised that this may be unrealistic and may result in reduced accrual to the bank as it is dependent on a number of issues, including pathology commitment and availability, theatre procedure and logistical issues. In general, the system established for collecting and transporting tissues for research should be developed around the specific operating policies of the institution (Grizzle et al 1998) – however when participating in a tissue bank network minimum standards must be met to ensure experimental results can be legitimately compared. This was the case at the Department of Pathology ERASMUS - the solution to the problem is detailed in **Case Study 1**.

Case Study 1 Department of Pathology Erasmus

Problem: Within the Department of Pathology, Erasmus it was discovered that the lag time from excision of tissue to snap freezing could - in exceptional cases - reach 3-4 hours.

Solution: A “quality circle” was established to discuss the aims of the tissue bank and pathology department protocols. All parties involved in the process were present at the meeting - in this case personnel of the operating theatres, logistics, the quality department, histology and the tissue bank.

A pilot protocol was then developed by the head of the tissue bank and the surgeon to study the process in three stages - within the operating theatre, during transportation and within the Pathology department. Areas where time could be saved were identified and implementation of the pilot protocol brought the lag time down to just under 30 minutes with clear areas highlighted where further work was needed. This included: the need for a general protocol for all operating theatres; the need for dedicated transport personnel; clear protocols for transfer of tissue from the operating theatre to the carrier and from the carrier to the pathology department; and clear protocols for pathologists and assistants for receipt of tissue.

Current situation: A budget has been allocated for a carrier and once hired the general protocol will be rolled out to all the operating theatres and clear protocols will be put in place within the pathology department. This will be followed up with an evaluation in a few months to assess the overall effect.

15. *Experimental data*

- In a time course degradation analysis of lung tissue Jewell et al (2002) found good nucleic acid stability was maintained for up to 5 hours after excision at room temperature. However, this has not been validated for other tissue types and further experimentation on the effect on gene expression profiles suggests that snap freezing tissue as soon as possible after it has been excised should be maintained as the ideal.
- A study by Spruessel et al (2004) to determine the impact of ischemia on gene and protein expression profiles of healthy and malignant colon tissue discovered that initial changes of gene

and protein expression profiles were already observed 5–8 min after colon resection. Fifteen minutes after surgery, 10%–15% of molecules, and after 30 min, 20% of all detectable genes and proteins, respectively, differed significantly from the baseline values.

- iii. Research by Huang et al (2001), also on human colon cancer specimens, aimed to quantitate the effects of warm ischemia – aliquots of tissue were frozen at times 5, 10, 15, 20, 40 and 60 minutes after excision. The conclusions that can be drawn from the microarray data presented is that ischemic times of less than 20 minutes provide relatively stable gene expression profiles, and that an ischemic time of more than 40 minutes results in significant deviations from baseline.
- iv. A time-course study on the differential gene expression of prostate tissue by Dash et al (2002) focussed on the fact that genes that appear up- or down-regulated could represent an artefact of RNA degradation with prolonged warm ischemia time. In the Departments of Urology and Pathology at University of Michigan most surgical specimens are routinely processed within 30 minutes of surgical extirpation, however 1 hour was taken as a realistic time frame for snap freezing. Experimental data showed that less than 0.6% of the more than 9000 genes tested were affected by an ischemic time of 1 hour at room temperature. However, some prostate cancer genes might be more susceptible to ischemia and all attempts should be made to process tissue rapidly to ensure that the microarray gene profile accurately reflects the state of the cells.
- v. Consistent with this is work carried out by Almeida et al (2004) on mouse liver to compare changes in mRNA expression levels and RNA stability in fragments of liver tissue exposed to 25°C and 37°C for specified times. At 25°C RNA degradation was limited, while at 37°C both the 18S and 28S rRNA species were affected, being almost completely degraded after 4 hours. Obviously the time under ischemic conditions at 37°C during surgery cannot be minimised, whereas the time at which samples are stored at room temperature in the period between excision and snap freezing can. However when mRNA expression level changes were assessed for seven genes, only one did not give a reliable estimate of in vivo mRNA levels through relative quantification of mRNA, even if performed on degraded RNA.
- vi. Once the sample has been frozen Taylor et al (1998) found that a technique allowing rapid extraction of RNA from individual frozen sections of prostate resulted in the RNA remaining stable at room temperature for up to 3 hours – this technique utilizes an RT-PCR compatible buffer solution containing RNAase inhibitor and dithiothreitol. If addition of the RNAase inhibitor was delayed and frozen sections were placed on unwetted glass slides and stored at room temperature for 4 or 10 days there was no decline in extracted mRNA after 4 days and a 22% decline after 10 days- this illustrates that once the samples have been frozen the resulting sections can be manipulated or stored at room temperature for a specified period.

Additional points

16. A recommendation made by the Advisory Panels for the development of the operational framework for the National Cancer Research Institute National Cancer Tissue Resource (NCRI NCTR) in UK is that tissue should be kept cool after excision by transporting it to the pathology department on ice, ideally within a plastic container/bag. It is suggested that this may delay degradative processes. Grizzle et al (1998) also advocate this approach and recommend the provision of a sterile bucket surrounded by wet ice in an insulated ice bucket positioned in the operating theatre.

To clarify, within the TuBaFrost consortium, tissue should be snap frozen within 30 minutes of excision from the patient. However, NCTR SOP Advisory Panels recommend the following for the NCRI NCTR in the UK and this may be considered for the TuBaFrost consortium - tissue subject to a delay of up to 2 hours could still be collected for the bank and the delay noted within the local database so that research recipients of the samples are fully informed about the quality status.

❑ DISSECT THE BIOPSY USING ASEPTIC TECHNIQUE

General

17. It is recommended that clean instruments are used for each resection and are cleaned or changed in between dissecting normal and tumour tissues.

Implementation

18. It is acknowledged that clearly displayed protocols will be necessary to ensure these procedures are embedded in routine pathology department practice of TuBaFrost collection centres. The use of foil and sterile instruments would be especially important if the tissue will eventually be used for RNA profiling.

❑ THE SUGGESTED SIZE OF TISSUE FOR SNAP FREEZING IS APPROXIMATELY 0.5CM³

General

19. The amount of tissue available will depend upon the sample site. It is recommended that duplicate samples should be collected if there is sufficient material.

Implementation

20. When tissue is to be stored in 2ml cryovials 0.5cm³ is the ideal size, however this is not so critical if cryomolds are used – at the CNIO Tumour Bank Network tissue fragments of 1.5 x 1 x 0.5 cm are frozen in cryomolds. The problem can be avoided by the use of the cryostraw system which facilitates the standardisation of the sample volume – further details of TuBaFrost work package 2 which looks at the logistics of collecting tissue using the cryostraw system is provided at **Annex D**. If only small fragments are available, they should still be collected for the TuBaFrost bank. Chu et al (2002) recommend the collection of tissue 5-10 mm in diameter. This is consistent with the recommendation of Grizzle et al (1998), which specifies the collection of small pieces of tissue, approximately 1 x 1 x 0.5 cm (or 0.5g).

Additional points

21. The histopathological diagnosis should not be compromised and areas of necrosis must be avoided. Within the Department of Pathology, ERASMUS further instruction and training has led to a 10 fold decrease in necrotic samples collected.

❑ THE TISSUE SAMPLES SHOULD BE FROZEN IN ISOPENTANE, EITHER DIRECTLY OR EMBEDDED IN A CRYOSOLIDIFIABLE MEDIUM.

General

22. Consultation with members of the TuBaFrost network and examples taken from other key tissue bank initiatives led to the recommendation that tissue samples collected for TuBaFrost should be snap frozen in pre-cooled isopentane (2-methyl butane). Isopentane is a very good cryoconductor and allows rapid freezing. In comparison to liquid nitrogen, it causes less damage during freezing as it remains in a liquid state so there are fewer cryo-artefacts. This contrasts to the freeze-boil effect observed when using liquid nitrogen. TuBaFrost does not recommend the use of liquid nitrogen as the freezing medium, nor slow freezing in a –80 °C freezer. However, if the cryostraw method is used the tissue (contained within the cryostraw) is frozen in liquid nitrogen (further details at **Annex D**).

To pre-cool the vessel of isopentane it should be suspended 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.

Care must be taken during the rapid freezing to ensure the sample does not crack. The use of a cryosolidifiable medium such as OCT is optional.

Discussions during meetings of the SOP Advisory Panels for development of the operational framework for the UK NCRI NCTR have suggested that to maintain protein integrity and ensure that frozen sections could be derived from the banked samples, samples should be frozen in isopentane, which is first cooled using cardice to form a cardice-isopentane slush. This allows greater accuracy of the freezing temperature.

Implementation

23. The methods described here have been implemented successfully in TuBaFrost participating institutions. Within the Department of Pathology, ERASMUS a slightly different method has been employed and as tissue quality is high this will be listed as an alternative freezing method in SOP 1.

ERASMUS freezing protocol:

Freezing the tissue samples is performed orientated, however not with OCT. The tissue sample is put on a piece of cork (approximately the same size as the tissue for support) along with an equal sized piece of Whatman paper soaked in physiologic salt solution. The site from which the sample for paraffin embedding is cut is directed upward and the paraffin sample is oriented with the cut site upwards in its block.

- USE A BAR-CODE SYSTEM FOR LABELLING THE SAMPLES; THIS WILL RESULT IN IMPROVED SAMPLE MANAGEMENT AND PRECISE IDENTIFICATION.**

General

24. The bar-code should be used in conjunction with the TuBaFrost code 'TF_institution code_sequential code' so that the sample is readable at institutions without access to a bar-code reader. The sequential code is the local inventory code and hence will not in any way relate to the pathology number or other identifiers. The sample is coded-linked so that key individuals with appropriate access rights are able to access other relevant datasets. In the absence of a bar-code system a waterproof pen and labels able to withstand storage at ultra-low temperatures should be used.

Implementation

25. In TuBaFrost institutes not already employing a bar-code system there has been no implementation of the system, due to the high prices involved to put this in place and the difficulty in changing long-standing methods. At ERASMUS a Laboratory Management System will be installed within the Department of Pathology in the future and this will mark a change over to a 2D bar-coding system. The vials will be labelled with the bar-code and text on the side of the vial and a text or barcode only sticker on the lid – the stickers and ink will be guaranteed durable for long term storage in liquid nitrogen and able to withstand sudden temperature changes.

ERASMUS recommends that vials marked with the local sequential code are not re-numbered upon issuing (i.e. addition of TuBaFrost institution code) but are simply accompanied by a minimal datasheet which gives the extra information necessary to identify the sample – this will be an option.

- ❑ **RECORD INVENTORY DETAILS IN A DEDICATED INVENTORY BOOK AND IN A PASSWORD PROTECTED ELECTRONIC INVENTORY DATABASE (WITH VARYING LEVELS OF ACCESS).**

General

26. The information to be recorded in the inventory (at a minimum) includes:

- TuBaFrost code (plus bar-code if in use);
- location co-ordinates;
- pathology number;
- type of tissue (both site and whether the tissue is tumour, normal or pre-malignant);
- time from excision to snap freezing;
- date of collection; and
- information re: potentially infectious material.

Implementation

27. This was either already in place or has been successfully implemented across the TuBaFrost institutes surveyed. Logging the movement and depletion of samples is yet to be implemented in some instances – this is an essential part of maintaining an accurate inventory.

- ❑ **STORE THE SAMPLE IN AN APPROPRIATELY SECURE AND MAINTAINED LIQUID NITROGEN BANK OR –80°C FREEZER. STORE DUPLICATE SAMPLES INDEPENDENTLY IF STORAGE FACILITIES ARE AVAILABLE.**

General

28. Key points

- ❑ Adequate maintenance – frost free, incident record book, temperature monitors, lockable repository.
- ❑ Alarm system – local alarms, central alarms and dial-out system.
- ❑ Contingency – repository of similar size and specification available for transfer of samples in the event of major breakdown, repository may be wired into hospital network (emergency generators)
- ❑ Cryovials should be stored in the vapour phase of liquid nitrogen or sealed in Cryoflex to avoid explosions.

Implementation

29. These recommendations have been generally successfully implemented across the TuBaFrost institutes surveyed. The Department of Pathology, ERASMUS provides a good example of existing repository management and successful implementation of certain points, detailed in **Case Study 2**.

CASE STUDY 2: Department of Pathology, ERASMUS

Back up: Back-up nitrogen system installed and hooked up to a central and automated filling system and to the alarm network.

Maintenance: Maintenance contracts in place and log books. No temperature monitors as liquid nitrogen in use and therefore lower risk of sudden thawing.

Security: long-term repositories are locked and keys are kept by designated tissue bank personnel.

Alarms: all storage facilities are connected to the alarm system - local alarm, central alarm and finally a dial-out system

Contingency: one empty but operational storage barrel is made available for transfer of samples in the event of major breakdown.

Health and Safety: Cryovials are stored under liquid nitrogen and all have a sealing ring in the lid to avoid explosions

The option exists to use either a liquid nitrogen bank or a -80°C freezer for the storage of samples for the TuBaFrost project. It is generally agreed that liquid nitrogen storage is recommended for proteomic research but for general use a -80°C freezer is adequate when coupled with appropriate risk management. Contamination issues regarding the use of liquid nitrogen and detailed by Burden (1999) can be avoided by using liquid nitrogen in the vapour phase – this is a Health and Safety Regulation in the United Kingdom due to the problem of cryovial explosions which result when trapped liquid nitrogen expands.

The subject of the dual storage of samples in different storage facilities was discussed at the TuBaFrost meeting in May 2004. It was felt that if adequate risk management is in place – for example a secure electricity supply back up for -80°C freezers – then duplicate storage is not essential.

Information regarding back-up strategies for other key initiatives includes:

- the SOP Advisory Panels for the UK NCRI NCTR recommend that dual storage is essential and that for a networked national archive of this size and significance the option of 3 storage banks should be considered - 1 working archive, 1 long term storage, and 1 long term storage back-up; and
- the UK Biobank (further information at www.ukbiobank.ac.uk) will be employing a system of dual storage, there will be a primary working archive in the form of a highly automated -80°C freezer and then a back-up liquid nitrogen archive. Dual storage should not be a strict criterion for new institutions joining TuBaFrost but will remain as a recommendation.

30. *Experimental data*

- i. Effective and secure long-term storage of the tissue samples is essential for the tissue bank network, evidence from Chu et al (2002) shows that in general the integrity and yield of DNA from gynaecological tissues remains unchanged with long-term freezing, whereas the integrity of RNA become compromised after storage of 5 years or longer and the yield became slightly lower and variable after 7 years.
- ii. Experience from the Biopathology Centres (BPC), Ohio, USA (Qualman et al, 2004) suggests that DNA and RNA yields will remain constant over a decade or more when tissue is stored long-term in vapour-phase liquid nitrogen freezers – this is the standard of storage at BPC.
- iii. According to Karlsson and Toner (1996) temperatures of or below -80°C are generally adequate for successful preservation of cells and tissues for extended periods of time and the shelf-life increases dramatically as the storage temperature is reduced. Mazur (1984) estimates that the shelf-life of cells stored at liquid nitrogen temperatures is 10³ years.

❑ **NEW INSTITUTIONS WISHING TO JOIN TUBAFROST WILL BE SUBJECT TO AN INITIAL QUALITY CONTROL ACCREDITATION.**

General

31. Quality assurance is fundamental to the successful operation of any repository that collects, processes, annotates, stores and distributes biospecimens for research purposes. Each collecting institute must be responsible for developing, managing, monitoring, evaluating, documenting and communicating its own quality assurance plan. Ideally a certified quality standard would be applied in each collecting institute under the overarching TuBaFrost network quality policy.

Elements of quality assurance for future consideration and development:

- Quality policy – an overview document which identifies the need for, and essential elements of, the quality system
- Specifications for biological resource materials and any primary testing (processing methods and equipment should be designed to deliver appropriate material and some form of validation performed to assure acceptable reliability/reproducibility)
- A process map for the work
- SOPs
- Use of traceable reference materials to enhance standardisation in quality of processing
- Other documents and forms recorded within the system
- Document control (archiving, review, amendment, storage etc)
- Staff training and competence should be documented and reviewed
- Auditing procedures to maintain standards and standardise processes performed in different centres

The initial accreditation will focus upon freezers, computer hardware, documented technical protocol, evidence of an informed consent document for tissue collection (if applicable in country of collection) and staff training records.

Implementation

32. Items for initial accreditation were generally considered to be acceptable across the TuBaFrost participating institutes. It was recommended that there should also be an assessment made of personnel availability and scientific interest of the pathologists. As outlined in Deliverable 7.1 and 7.2, evidence of an informed consent document is only required when the national law in a specific country dictates this, and thus TuBaFrost SOPs should be flexible to reflect this.

Adequate information and training is essential in a large tissue bank network, staff of the Cooperative Human Tissue Network, USA - which has six divisions and distributes approximately 80,000 research specimens to researchers annually – undergo annual training at the Biopathology Centres (BPC), Ohio with particular focus upon timely snap-freezing of tissue (Qualman et al, 2004). Feedback from researchers on the quality of RNA and DNA supplied allows annual verification of institutional performance.

❑ DURING YEAR 1 THERE SHOULD BE A QUALITY CONTROL REVIEW OF 2% OF NEW CASES TWICE A YEAR.

General

33. In the first year of collection an institute should be subject to a 6-monthly quality review of 2% of newly collected samples. In the absence of issues encountered during the first year of collection, this should be reduced in subsequent years to 1% of new cases received annually. The review will focus upon records, equipment, frozen section and fixed sections. Fixed sections will not routinely be collected for TuBaFrost, however this may be necessary to provide an additional level of quality control and prevent the waste of frozen tissues.

Implementation

34. This quality system has worked successfully for the distributed network of the CNIO Tumour Bank Network, Madrid. Establishing a quality system may be a challenge for some institutions but with clear guidelines it can only improve the quality of tissue collected and increase the potential collaborations for the institute. It was suggested that the regularity of review should depend upon the number of tissues banked, for example if there are more than 200 samples collected during the first 6 months then the review will take place twice per year.

- ❑ **THE QUALITY CONTROL OF THE FROZEN SAMPLES WILL FOCUS UPON SAMPLE IDENTIFICATION, REVIEW OF STAINED H&E SECTIONS AND QUALITY OF EXTRACTED DNA.**

General

35. Whilst checking the sample identification and location, the durability of the sample vessels and the inventory containers can also be checked to ensure they have remained stable at low temperatures. The stained H&E sections will be reviewed by a pathologist to confirm the diagnosis and assess how representative it is of the sample. The quality of the extracted DNA will be checked using an agarose gel or a bioanalyser – the bioanalyser requires less material and provides integrity and concentration information.

Implementation

36. The quality control procedure needs further validation through work with the CNIO Tissue Bank Network. Generally the system proposed is viewed as a sound proposal for quality control and in some instances the H&E check already takes place on all samples collected.

- ❑ **BLOCK AND SLIDES SHOULD BE STORED UNDER APPROPRIATE CONDITIONS TO PREVENT DEGRADATION AND THE INVENTORY SYSTEM SHOULD BE SECURE AND MAINTAINED.**

General

37. Key points:

- Climate controlled room (temperature and humidity) or refrigerator
- Controlled exposure to direct sunlight
- Frozen sections stored in a freezer
- Slides can be stored under a protective layer of paraffin, under vacuum or under gaseous nitrogen
- Inventory system – ordered filing system, light exposure controlled, lockable repository, controlled access.

Implementation

38. Across the TuBaFrost network, blocks and slides produced for routine diagnostic purposes are generally kept in a controlled access store and there is a system in place for lending and issuing blocks or it is under the control of the Laboratory Management System. The blocks are not routinely stored in a climate controlled room, except in certain institutes.

REFINED STANDARD OPERATING PROCEDURES FOR THE TUBAFROST PROJECT:

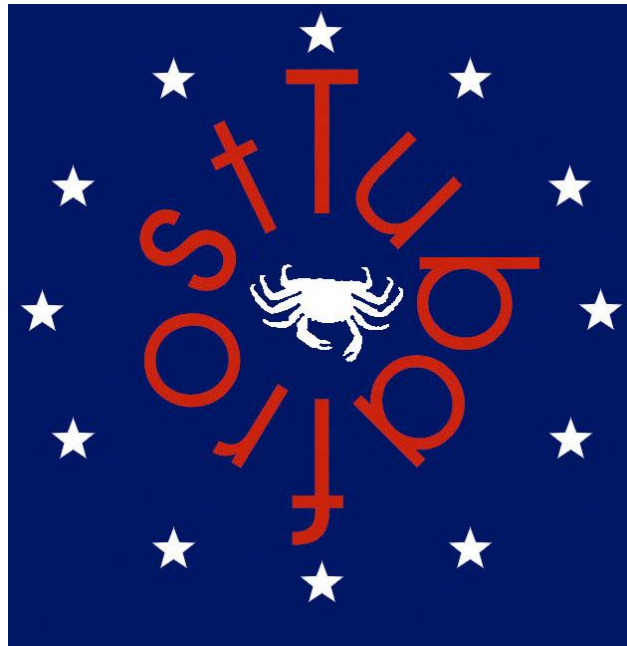
39. The remainder of this report sets out the refined TuBaFrost Standard Operating Procedures for:

- SOP 1: The collection of human tumour and corresponding normal tissue;
- SOP 2: The storage of human tumour and corresponding normal tissue; and
- SOP 3: The quality control policy for collection and storage of tissue.

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TuBaFrost Standard Operating Procedure 1

Collection of human tumour and corresponding normal tissue
for the European Human Frozen Tumour Tissue Bank

Version 2 Updated 23/08/04

PURPOSE

This Standard Operating Procedure (SOP) defines the collection procedure of human tumour and corresponding normal tissue for the European Human Frozen Tumour Tissue Bank (TuBaFrost). Frozen tissue samples are used in a wide variety of experimental techniques and when sourced from a distributed network there must be conformity in tissue freezing techniques to ensure results from these experiments are reproducible and comparable.

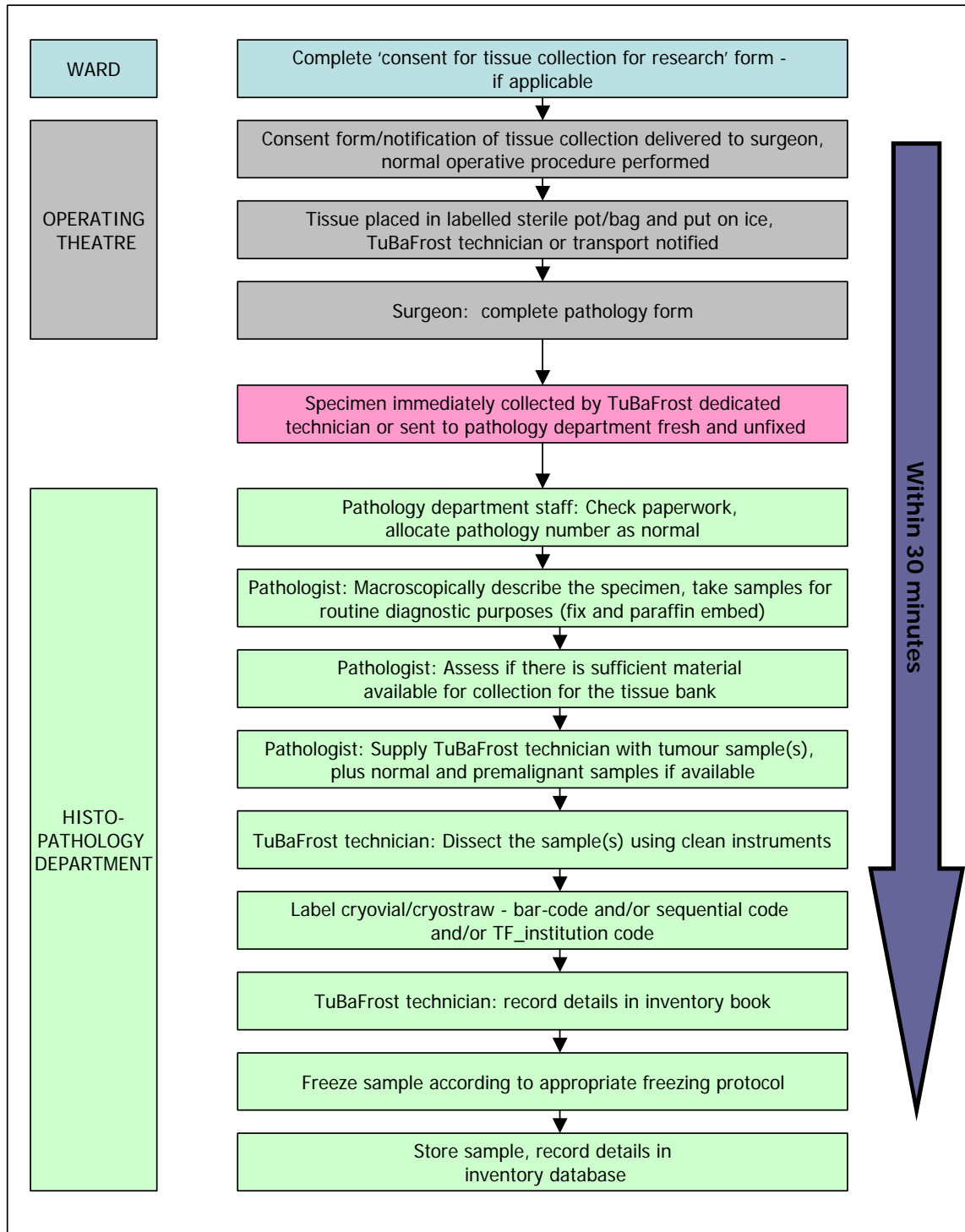
SAFETY

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

ASSOCIATED DOCUMENTS

Consent form if applicable (according to national law in collecting country)
S.O.P. 2 v. 2 Protocol for storage of human tumour and corresponding normal tissue

WORK FLOW FOR COLLECTION OF TISSUE



WARD

- 1.1 Consent taken from patient (if applicable – according to the law in the collecting country)

OPERATING THEATRE

- 1.2 Deliver notification of tissue collection (and consent form if applicable) to surgeon or flag up on operating list

Surgeon

- 1.3 Perform normal operative procedure, record time of excision of specimen
- 1.4 Place specimen in labelled sterile pot/bag and put on ice
- 1.5 Complete pathology form

Operating theatre staff

- 1.6 Notify TuBaFrost dedicated technician or transport service – send specimen immediately to pathology department fresh and unfixed

HISTOPATHOLOGY DEPARTMENT

- 1.7 Notify pathologist and dedicated TuBaFrost research technician (if not already present)
- 1.8 Check paperwork and allocate pathology number to specimen as routine

Pathologist

- 1.9 Macroscopically describe specimen as routine
- 1.10 Using clean instruments and on a clean surface (sterile foil or clean dissection board) dissect the specimen – clean or change instruments between dissecting normal and tumour tissue
- 1.11 Take representative parts for routine diagnosis (for fixation and embedding) as priority and decide if there is sufficient material available for the tissue bank
- 1.12 Supply research technician with sample(s) for cryostorage - representative parts of the lesion, normal tissue and pre-malignant conditions

TuBaFrost Technician

- 1.13 Prepare the sample for snap freezing on a clean surface and using clean instruments - change instruments in between preparing normal and tumour tissue. The minimum size of tissue for snap freezing is approximately 0.5cm³ though the amount of tissue available will differ depending upon the sample site. Smaller fragments should still be snap frozen for the tissue bank. If there is sufficient material freeze duplicate samples - therefore there may be many samples per biopsy.

- 1.14 Options for preparation of freezing medium:

A. 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.

B. Prepare the freezing medium by adding dry ice (cardice) to the isopentane until a slush is formed.

- 1.15 Label cryovials, cryomolds or cryostraws with a bar-code and/or sequential code and/or TF_institution code (depending upon local laboratory practice). Use waterproof pen able to withstand long-term storage at low temperatures. The sequential code is the local inventory code and must not relate to the pathology number or other identifiers. If a bar-code is used the TuBaFrost code must also be included to make the sample identifier readable at institutes where there are no bar-code readers. Alternatively a sheet must be supplied with the samples when they are distributed to relate the bar-code to the TuBaFrost code or the sequential code to the full TuBaFrost code.
- 1.16 Record the local sequential code, pathology number, date, lag time from excision to freezing, the type of tissue (site and whether the sample is tumour/normal/premalignant) in the inventory book. If a bar-code system is in use this can be scanned into the Laboratory Management System and the above data recorded.
- 1.17 Options for freezing:
- A.** Embed the tissue samples in O.C.T. (optimal cutting temperature) compound and freeze in isopentane or freeze directly in isopentane. The isopentane used is either cooled by suspension in liquid nitrogen or through addition of dry ice. Do not remove the tissue from the isopentane until freezing is complete (5 seconds or less depending on size) but ensure sample does not crack. Remove sample from isopentane and enclose in the labelled cryovial.
- B.** Orientate the tissue on a piece of cork and an equally sized piece of Whatman paper soaked in physiologic salt solution. The isopentane used is either cooled by suspension in liquid nitrogen or through addition of dry ice. Do not remove the tissue from the isopentane until freezing is complete (5 seconds or less depending on size) but ensure sample does not crack. Remove sample from isopentane and enclose in the appropriate labelled storage vessel.
- C.** Embed samples in a cryosolidifiable medium in plastic cryomolds and immerse in the pre-cooled isopentane. The isopentane used is either cooled by suspension in liquid nitrogen or through addition of dry ice. Do not remove the tissue from the isopentane until freezing is complete (5 seconds or less depending on size) but ensure sample does not crack
- D.** If the cryostraw system is used introduce a carrot of tissue into the straw, thermally seal each extremity and place in liquid nitrogen.

All tissue must be snap frozen within 30 minutes of excision from patient. Tissue subject to a delay of up to 2 hours should still be collected and the delay noted within the local inventory database.

- 1.18 Follow standard operating procedure 2 for storage guidelines.



TuBaFrost Standard Operating Procedure 2

Storage of human tumour and corresponding normal tissue for
the European Human Frozen Tumour Tissue Bank (TuBaFrost)

Version 2 Updated 23/08/04

INTRODUCTION AND PURPOSE

This Standard Operating Procedure defines the storage procedure of human tumour and corresponding normal tissue for the TuBaFrost project.

SAFETY

Carry out all procedures in accordance with the local codes of practice.

Working with liquid nitrogen is hazardous - therefore all procedures should comply with local safety rules specific to this chemical.

All tissue must be handled as if potentially infectious.

ASSOCIATED DOCUMENTS

S.O.P. 1 v. 2 Protocol for collection of human tumour and corresponding normal tissue

PROCEDURE

1.1 Options for storage

A. Transfer the snap frozen sample from the isopentane to a pre-chilled storage container for transfer to either a locked -80°C freezer or liquid nitrogen storage facility in liquid or vapour phase.

B. Place cryostraws in a designated visiotube within a goblet (removable liquid nitrogen storage elements) and place within the locked liquid nitrogen repository.

Store duplicate samples in a different storage facility if this is available.

1.2 Check the alarm system of the storage repository. This should be 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 and c) a remote alarm capable of automatically dialling out pre-programmed telephone numbers.

1.3 Check the back-up system for the storage repository - either a back-up freezer running constantly or adequate supplies of liquid nitrogen.

1.4 Record storage details in the inventory book and check earlier data that was entered. At a minimum the information recorded will include: inventory number (local sequential code); location co-ordinates; pathology number; type of tissue (site and also whether the

sample is tumour/normal/premalignant); lag time between excision and freezing; and date.

- 1.5 Transfer details to the computerised database system.
- 1.6 Update the database when samples are moved or depleted.



TuBaFrost Standard Operating Procedure 3

Quality assurance of tissue collected for the European Human Frozen Tumour Tissue Bank (TuBaFrost)

Version 2 23/08/04

Quality Assurance is fundamental to the successful operation of the European Human Frozen Tumour Tissue Bank (TuBaFrost) and important elements include written standard operating procedures, quality indicators (quality control) and quality objectives.

Each collecting institute must be responsible for developing, managing, monitoring, evaluating, documenting and communicating its own quality assurance programme. Ideally a certified quality standard would be applied in each collecting institute. The local quality assurance programme will be part of the overarching TuBaFrost quality assurance policy.

TuBaFrost network quality control

- Records
- Appropriate SNOMED codification (if centralised)
- Registries of activity across the network

TuBaFrost local quality control - initial accreditation of institutes joining the TuBaFrost network

- Equipment
 - Freezers with security supports
 - Hardware
 - Secure and private telephone line (ISDN)
- Technical documented protocols
- Ethics (if applicable by law in collecting country)
 - Informed Consent document
 - Ethical approval from appropriate authorities

Ongoing local quality control

During the first year of collection for TuBaFrost 2% of new cases collected in an institute will be reviewed twice per year. If no problems emerge in the first year the review will be reduced to 1% of new cases annually.

Quality control will focus upon:

- Records and files
 - Appropriate informed consent (if applicable)
 - Minimum datasets which an acceptable file must contain: tumour stage, grade, size, localization sex, age

- Appropriate SNOMED coding
 - Records about requests, activity, incidences.
- ❑ Equipment
- Technical maintenance (protocols)
 - Distribution of samples into freezers
- ❑ Review of 2% of the new cases, twice a year
- Cases must be selected at random but only from the common tissue types (colon, breast, lymph nodes, lung, appendix and tonsil). Do not select rarer types (brain, skin)
 - Frozen tissue
 - Review of the sample identification (bar code, labelling)
 - Reviews of stained HE sections by a pathologist in order to assess/ confirm the diagnosis and how representative it is of the sample
 - RNA extraction (Trizol) for RNA quality assessment in an agarose gel (two bright bands 28S and 18S rRNA)
 - Or use the Agilent Bioanalyzer system. This requires only a small amount of RNA (50-100ng) and yields information about integrity and concentration.
 - Fixed tissue (not specifically collected for TuBaFrost but available through routine diagnostics – could provide a further level of quality control)
 - Review of stained HE sections by a pathologist in order to assess/ confirm the diagnosis and if the sample is representative of the process
 - Checking the sample identification (bar code, etc)
 - Test of fixation: immunohistochemical staining to evaluate optimal sample fixation (antigen preservation) in paraffin blocks. Antibodies used: Ki67, CD34. Vimentin
 - It is very useful to use a tissue-microarray (1,5 mm in 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 and lowers costs.

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Quality of Life and Management of Living Resources

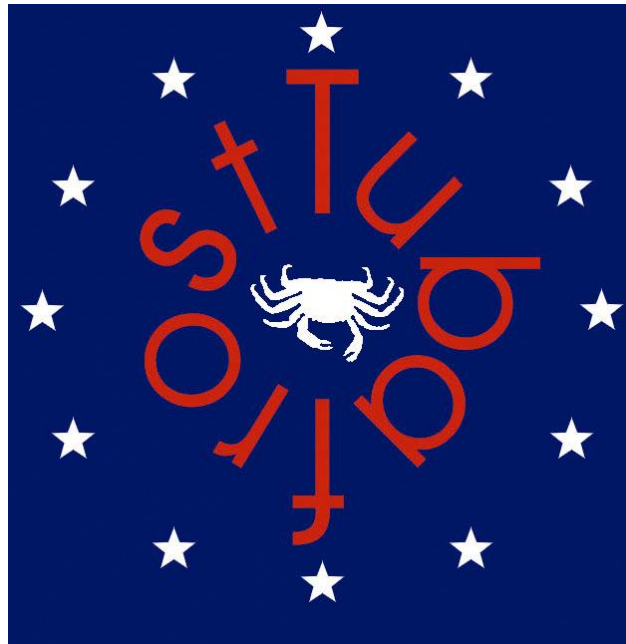
European Human Frozen Tumor Tissue Bank

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

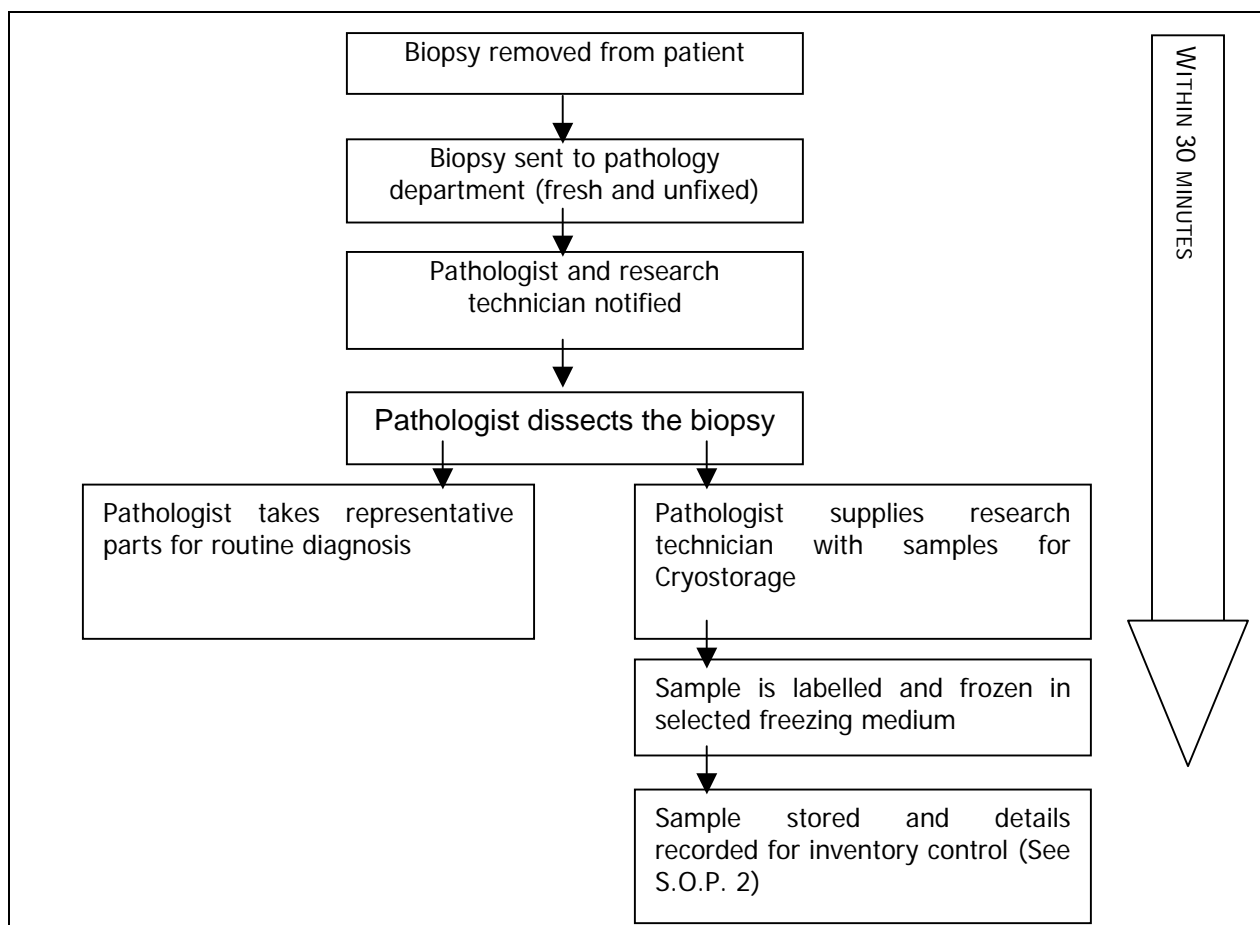
Carry out all procedures in accordance with the local codes of practice. Working with liquid nitrogen and isopentane is hazardous therefore all procedures should comply with local safety rules specific to these chemicals. All tissue must be treated as potentially infectious.

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 embeds 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.

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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 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.

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The BOC group (hazard data sheets) www.boc.com



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

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, tumoral 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 representatives of the samples.
 - 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 analyser.

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

Annex B

Quality of Life and Management of Living Resources

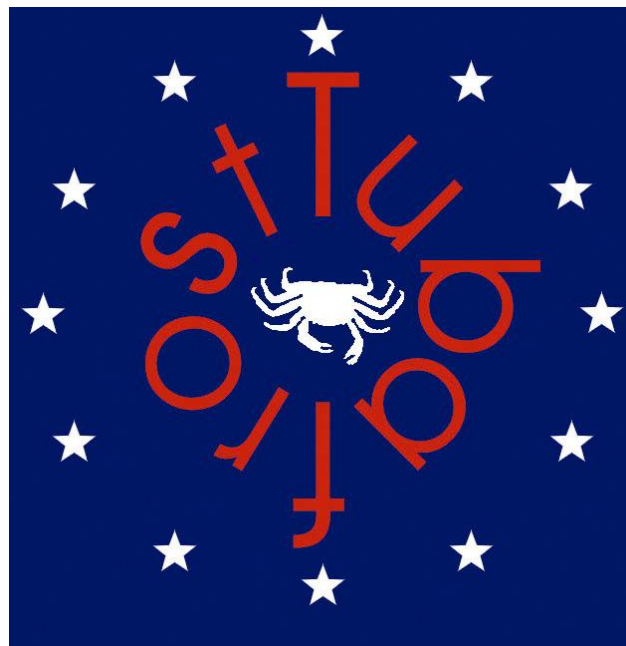
European Human Frozen Tumor Tissue Bank

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Deliverable D 3.2

Basic design of the local system for storage of human tumour
and corresponding normal tissue



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**TUBAFROST Work Package 3: Basic design of the local system for storage of human tumour and corresponding normal tissue
(Deliverable 3.2 November 2003)**

This deliverable builds upon the storage aspects of the recommendations made in Deliverable 3.1 'Protocols and systems for the collection and storage of tissue'. These recommendations were made following research into current best technical practice across the TuBaFrost consortium. This deliverable deals specifically with storage, which is an essential if basic element of the TuBaFrost operational framework and contributes to the ultimate aim that research using TuBaFrost tissue must yield reliable and reproducible results. This can only be attained by the training and informing of all personnel involved with tissue bank activities and a focus on the key points:

- Barcodes
 - technical specification
 - information recorded

- Inventory systems
 - technical specification of sample vessels and inventory containers
 - quality control of sample vessels, inventory containers and data storage

- Storage repositories
 - Liquid nitrogen and -80°C freezers
 - technical specification
 - maintenance
 - temperature monitoring
 - associated hazards
 - multiple storage sites

- Security measures and contingency plans
 - alarms
 - back-up
 - biosecurity

- Storage of blocks and slides
 - environment
 - inventory system

Barcodes

Results from research into sample labelling methods (carried out for Deliverable 3.1) showed that the majority of TuBaFrost participants use waterproof permanent pen able to withstand long-term storage at low temperatures. While this is a valid and commonly used method within and beyond the TuBaFrost network the use of barcodes is recommended as it improves the accuracy of sample identification and can facilitate sample management and distribution. The technical specification of the labels is also relevant beyond just barcodes as some participants use the waterproof pen on labels.

- technical specification
 - label and ink durability
 - barcode printers and scanners
- information recorded
 - barcode and human readable text

Technical specification^{1, 2}

Label and ink durability

For frozen tissue samples:

- the label and its adhesive must be able to a) withstand a wide range of temperatures (the minimum being approximately -196°C boiling point of liquid nitrogen) b) withstand an archival life of many years at extremely low temperatures and c) be self adhesive on many different materials;
- it is essential that the label remains firmly affixed and legible;
- ideally the tissue will not be subjected to repeated freeze-thaw cycles but in the event of this the label must be able to resist moisture;
- the label should be tamper-resistant i.e. any attempt to remove it will result in its destruction;
- handling problems may be overcome by selecting labels with adhesives more compatible to working with laboratory gloves;
- the ink on the label must be quick drying to prevent smudging when applied to the sample vessel; and
- the label must be adhesive even when applied to cold/damp surfaces.

For blocks and slides:

- the label and its adhesive should be able to withstand stains and solvents using xylene;
- the label and its adhesive should be able to withstand cold temperatures; and
- the label and its adhesive must have a long archival life.

Barcode printers and scanners

Using the specified durable ink the barcode labels can be printed in house or brought in pre-printed. Regular tissue sample vessels (cryotubes, cryomolds, cryostraws, etc) will require relatively small labels so the printer must be of high quality and specific for the task. For some tissue banking locations it may be useful to have wireless equipment to allow printing from anywhere within the institute/hospital. Consideration should be made of the laboratory environment in which the printer will be used, a casing resistant to water, chemicals or body fluids may be necessary. For extra security samples can be double labelled with exactly the same barcode, this especially applies to paraffin blocks where the barcode can be labelled on the cassette and also affixed to the paraffin (as in Centro Nacional de Investigaciones Oncologicas), of course the option also applies to affix the bar-code to relevant paperwork.

Scanners can be hand-held or fixed and use cable or cable-less data collection. The size of the bar code should be taken into consideration and also the environmental conditions, when retrieving a sample from a freezer there may be frost build-up obliterating the barcode. Many scanning devices will not tolerate labels with even partially unreadable barcodes or barcodes printed in colours other than black and white. For sophisticated applications the label can be scanned and this will automatically allocate a storage location within the repository.

Information recorded

Sample management can be greatly improved by considering the label layout and design. Early decisions to be made include:

- standard layout for the label;
- data identifiers for barcodes used;
- the data structures that carry information i.e. how a particular barcode will be recognised by the reader, how many characters there are and whether the characters are letter, numbers or both; and
- technical details for the barcode itself, such as minimum and maximum heights and widths of bands.

Results from the questionnaire circulated for Deliverable 3.1 revealed that if institutes were using barcodes then these were generally in conjunction with human readable text (as in Centro Nacional de Investigaciones Oncologicas). This means that samples can be easily located through this human readable text (in the case of TuBaFrost 'TF_institution code_local code') and the text should be of a reasonable size, as should the barcode to allow efficient reading by the scanner. Other less frequently referenced information can be quite small though as generally agreed by the TuBaFrost consortium there should be no identifiers (e.g. pathology number, patient name) included on the sample vessel, slide or block.

Inventory systems

- technical specification
 - sample vessels
 - inventory containers
- quality control
 - sample vessels and inventory containers
 - data storage

Technical specification

Sample vessels

Cryovials, cryomolds or other storage vessels (e.g. cryostraws) used for storing tissue for the TuBaFrost tissue bank must be:

- specifically designed for storing biological materials at temperatures as low as -190°C;
- stable when submitted to sudden low temperatures (snap freezing), when held at low temperatures for long periods of time (years) or when taken through several freeze-thaw cycles; and
- as leak proof as possible (applicable to cryovials) even at the lowest cryogenic temperatures.

Inventory containers

Inventory containers and systems used vary across the TuBaFrost consortium but all must be:

- specifically designed for storing biological materials at temperatures as low as -190°C;
- stable when submitted to sudden low temperatures (snap freezing), when held at low temperatures for long periods of time (years) or when taken through several freeze-thaw cycles. Metal (aluminium or stainless steel) drawer racks and shelves and polycarbonate boxes are versatile and hardwearing in -80°C freezers and liquid nitrogen repositories. Fibreboard boxes are also commonly used in -80°C freezers;
- specifically designed for the size of the sample vessels otherwise the sample vessels may be damaged and the labels scratched off.

Quality control

Sample vessels and inventory containers

Quality control of the sample vessels and inventory containers is relatively simple but very important, if samples are incorrectly identified there is no point retaining them in the tissue bank. A periodic review of the identification system used is essential, both to ensure identification is accurate and to ensure the identification label or writing has not been damaged. As detailed previously, the accuracy of an identification system can be greatly improved by the use of bar codes with connections between the scanner and the database computer. Also, a check should be made to ensure that the sample vessels (cryovials or cryomolds) and inventory containers are not becoming brittle through long-term storage at low temperatures.

Data storage

A recommendation made in Deliverable 3.1 was for the double entry of data, firstly into an inventory book and then onto the local database, it is recommended that samples within the bank are randomly checked against the data in the inventory book and on the database to ensure they are in the correct locations. A further recommendation was that the database should be regularly updated as samples are moved and exhausted and this would also ensure that redundant spaces are re-allocated within the repository. The data inventory must be regularly

backed-up and only accessible by registered users who will have varying rights of access to the data.

Storage repositories

A major decision arising from discussion and feedback from Deliverable 3.1 was that for economical reasons it would be left up to each TuBaFrost participant to decide whether to use a liquid nitrogen facility or -80°C freezer. Many scientists think that storage at lower temperatures helps preserve the integrity of the specimen for long-term storage,^{3,7} however there is no general consensus on this. A further possibility is the Cryogenic storage freezer which provides mechanical convenience with cryogenic temperature performance (-140°C and -150°C) and uniform temperatures throughout⁴.

The main aims of any storage repository should be to provide the user with a fully automated, safe and reliable storage system through consideration of the following elements:

- technical specification;
- maintenance;
- temperature monitoring;
- associated hazards; and
- multiple storage sites

Technical specification (key features)

When setting up a tissue bank it is necessary to assess the required capacity of the storage repository. This can be done by looking at past records of collection or surgical activity and allocating space accordingly or purchasing a dedicated repository capable of supporting the tissue bank activities in the long term. The storage vessels and inventory containers used will have a major effect on capacity required, depending upon whether 2ml cryovials, cryomolds or cryostraws are used.

For a liquid nitrogen repository auto feed of the liquid nitrogen is desirable but non-essential if an adequate alarm system is employed.

A low profile design is recommended for ease of access to inventory or alternatively the positioning of a lifting device close to the repository should be considered. This is especially relevant to the hazard of oxygen depletion related to the use of liquid nitrogen repositories- users must be made aware that there is an oxygen-deficient atmosphere inside large storage containers. Care must be taken to ensure that people retrieving samples cannot lean over the containers in such a way that they might breathe this atmosphere and collapse into or over the container, resulting in asphyxiation.

Maintenance

Weekly/Monthly/Annual maintenance plans

Weekly or as needed: ensure storage inventory systems are maintained frost-free and undamaged, check storage repository for ice build up especially around the door seals. Check temperature alarms and oxygen depletion alarms and their batteries.

Monthly: change/clean filters

Annual: shut down and deep clean

Annual: maintenance contract with dedicated company focussing on re-calibration and validation through a temperature test carried out on various points throughout the repository.

It is also recommended to maintain an incident book both for recording maintenance events and for faults.

Temperature Monitoring

A major recommendation made in Deliverable 3.1 was the need for all storage repositories to be fitted with an alarm system, this is detailed in the next section 'Security measures and contingency plans'. Beyond the alarm system it is also recommended that the temperature of the repository is constantly monitored locally through weekly/monthly graphs. These real-time temperature monitors are generally supplied separately to the repository. As an alternative morning and evening temperatures should be logged and the temperature checked and alarms attended live throughout the day.

To prevent large temperature variations when the repository is open (relevant to -80°C freezers) the door should not be opened for longer than 30 seconds. When sourcing samples only 1 tray should be removed at a time and the specimens should be placed straight onto ice.

Associated Hazards

For liquid nitrogen repository and -80°C freezer:

- skin contact with liquid nitrogen or cold nitrogen gas may cause severe cold burns;
- unprotected skin may freeze onto cold surfaces, causing severe damage on removal;
- prolonged skin exposure to cold may result in frostbite;
- prolonged inhalation of cold vapour or gas may cause serious lung damage; and
- splashes of liquid nitrogen, or short exposures to cold vapour or gas, may cause instant freezing of eye tissues and permanent damage.

Recommendations:

- use adequate Personal Protective Equipment (PPE), specifically
 - gloves designed for purpose (cryo-gloves);
 - goggles or face shield;
 - no open-toed footwear; and
 - lab coat/overalls and cryo-apron.

For liquid nitrogen repository hazards⁵:

- large volume of gas produced on evaporation;
- low temperature;
- low viscosity means that it rapidly and completely covers surfaces on which it is spilt; and
- on boiling, liquid nitrogen produces approximately 700 times its volume of gas. The resulting displacement of oxygen from the atmosphere may be sufficient to cause asphyxiation if it occurs in a confined space.

Recommendations:

- adequate ventilation;
- where adequate ventilation is insufficient to control the build-up of nitrogen gas, or where leaks or spills would reduce the oxygen content to below 18 vol %, it is recommended that fixed oxygen monitoring equipment must be used; and
- the alarms triggered must be visible and/or audible both inside and outside of the area monitored, in order to give adequate warning of oxygen depletion. These must be regularly checked.

For storage of cryovials in liquid nitrogen:

There is currently no screw top cryogenic vial on the market today that can claim to be leak proof in liquid nitrogen so the following recommendations are particularly important^{4,6}. If liquid nitrogen is trapped inside a container that is sealed, then expansion on warming above -196°C may cause

an explosion, giving rise to danger from contamination by the vessel's contents as well as injury from fragments of the vessel itself.

Recommendations:

- make staff aware of potential risks;
- use vapour phase of liquid nitrogen;
- if using liquid phase
 - ensure vials are adequately sealed before placing in repository; and
 - seal cryovial in CryoFlex (plastic tubing that is sealed around the tube)
- when removing existing samples from the liquid phase
 - wear a face shield; and
 - immediately place samples into a secondary container with a closed lid to warm up or store for 24 hours in the vapour phase.

Multiple storage sites

Even with adequate back-up and alarm systems it is recommended to have duplicate tissue samples stored:

- in a separate freezer in the same facility; or ideally
- in an off-site facility with separate electricity supply.

□ Security measures and contingency plans

Alarms

The storage repository must have an alarm network in place. As recommended in Deliverable 3.1 the TuBaFrost project standard should be a tri-phase alarm system with

local visual and acoustic alarms where the storage repository is located; a distant acoustic and visual alarm in a central surveillance facility; and a remote alarm capable of automatically dialling out pre-programmed telephone numbers of on-call personnel.

To prevent unnecessary call outs there should be a delay mechanism in place, the local alarm would have to sound for a specified amount of time before triggering the distant acoustic and visual alarm. There should be an emergency contact list clearly displayed in the freezer location. It is essential to test the functioning of the alarms during routine maintenance and to regularly check the batteries. Surge protectors fitted to the storage repositories would also provide some protection against unnecessary activation. In some countries an alarm may be necessary if room temperature is likely to affect function.

Back up

Around the clock monitoring and weekly/monthly/annual maintenance programmes are vital for ensuring specimens are maintained at the necessary temperature. However, there is always the risk of major mechanical breakdown or power failure and so the following recommendations are made:

- inclusion in a secure electricity supply backed up by emergency generators;
- maintain empty freezer space of similar dimension and characteristics close to the repository (also useful during cleaning of main equipment);
- maintain easily accessible liquid nitrogen source for liquid nitrogen repository; and
- maintain a supply of carbon dioxide for –80°C freezers back up. This can be built in or freestanding and works by injecting liquid CO₂ into the cabinet when the temperature warms to a pre-set level.

Biosecurity

Tissue banks are valuable resources that must only be used by well-informed and trained individuals. It is therefore recommended that freezers:

- are lockable;
- have tamper resistant function selectors; and
- have key-operated main power on/off switch.

The inventory database should:

- only be accessible by certain individuals; and
- have password controlled rights of operational access

Contamination is an issue with liquid nitrogen repositories⁴ as the liquid nitrogen will not only serve as a refrigerant but also as a vehicle for transmission of viruses, bacteria, fungi and animal cells. As previously discussed no cryovial is 100% leak proof when submerged in liquid nitrogen and this can cause contamination both of the sample and the liquid nitrogen. It is recommended that the liquid nitrogen is periodically checked for contamination and that the use of Cryoflex or other material is considered as an option.

Storage of blocks and slides

Research for Deliverable 3.1 showed that almost all of the participants store tissue as blocks and slides as well as frozen and while this work package mainly deals with the storage of frozen tissue there are a few basic recommendations that may be relevant.

Environment

The recommendations are to consider storing blocks and slides

- in a climate controlled room (temperature and humidity);
- in a refrigerator;
- with controlled exposure to direct sunlight (especially for stained slides or paraffin blocks);
- in a freezer (relevant to slides), this is especially important for frozen sections

Further options exist for slides (especially for tissue array slides)⁷, such as

- to store sections for a long period of time under a protective layer of paraffin to maintain immunohistochemical activity;
- to store slides that are accessed frequently under vacuum; or
- under gaseous nitrogen for long-term storage.

Inventory system

It is recommended that the inventory system for blocks and slides

- uses an ordered filing system dependent upon human readable data if no barcode scanner is available;
- makes use of a dedicated slide storage cabinet will also control exposure of slides to light;
- uses a lockable storage system; and
- creates controlled access to blocks and slides, if items are removed they must be logged.

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Quality of Life and Management of Living Resources

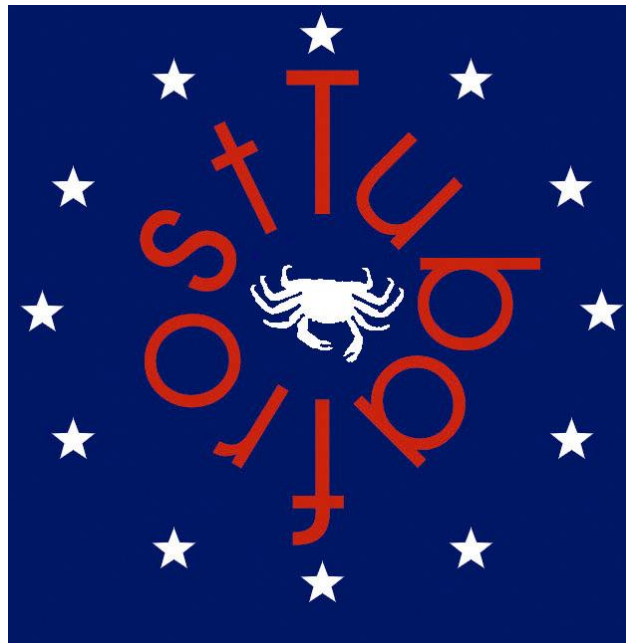
European Human Frozen Tumor Tissue Bank

TUBAFROST

QLRI-CT-2002-01551

Deliverable D 3.3

**Implementation of a local system for collection and storage of
human tumour and corresponding normal tissue
DETAILED FEEDBACK ON DELIVERABLE 3.3 AND MILESTONE 3.1
QUESTIONNAIRE**





Deliverable 3.3 Implementation of a local system for collection and storage of human tumour and corresponding normal tissue

In order to validate the standard operating procedures (SOPs) and recommendations made in Deliverable 3.1 and 3.2 a questionnaire was circulated to TuBaFrost participants. As the implementation of the SOPs and recommendations was a multi-centre effort details were requested about where a certain procedure (whether new or well-established) had worked effectively and equally where it had been difficult or difficulties were foreseen. It is essential that new collectors joining the TuBaFrost project are supplied with clear and concise SOPs, which reflect best practice and are usable within a hospital environment. The SOPs must also be as current as possible, reflecting recent changes in technology and publication of experimental data relevant to this area of tissue banking, therefore participants were also requested to indicate any areas where this is the case and updates are required.

TuBaFrost members not directly involved in tissue collection and storage were also asked to indicate whether the recommendations were in-keeping with their work packages (for example, ethically sound) and if they were content for collection and storage of TuBaFrost material to follow these procedures. Feedback from all the participants will be discussed within the report for Milestone 3.1 'Demonstration of the working standardised local storage system, start the actual tissue collection and report'. Only through the validation of these SOPs and recommendations can tissue collected for TuBaFrost be used to produce reliable and reproducible research results.

Key recommendations highlighted for discussion and validation are:

- Establish an organisational structure for the efficient collection and storage of tissue;
- The specified time limit from excision of tissue to snap freezing is 30 minutes;
- Dissect the biopsy using aseptic technique;
- The suggested size of tissue for snap freezing is approximately 0.5 cm²;
- The tissue sample should be snap frozen in isopentane, either directly or embedded in a cryosolidifiable medium;
- Use a bar-code system for labelling the samples;
- Record inventory details in a dedicated inventory book and in a password protected electronic inventory database;
- Store the sample in an appropriately secure and maintained liquid nitrogen bank or -80°C freezer. Store duplicate samples independently if storage facilities are available;
- New institutions wishing to join TuBaFrost will be subject to an initial quality control accreditation;
- During Year 1 there should be a quality control review of 2% of new cases twice per year;
- The quality control of the frozen samples will focus upon sample identification, review of stained H&E sections and quality of extracted RNA;
- Blocks and slides should be stored under appropriate conditions to prevent degradation and the inventory system should be secure and maintained.

Feedback on implementation of recommendations made within Deliverables 3.1 and 3.2

TuBaFrost recommendation: Establish an organisational structure for the efficient collection and storage of tissue.

Key points: Communication with operating theatre and pathology department staff. Biopsy removed in theatre and sent immediately to pathology department fresh and unfixed, pathologist and dedicated TuBaFrost technician immediately notified. Pathologist selects tissue for diagnosis as priority and if there is sufficient material then duplicate samples can be frozen.

Has this been implemented in your institution?

Yes (ERASMUS)

Yes (Valencia Institute of Oncology)

Yes. The pathology facility for frozen and fresh material is within the block of the operating theatre. The presence of the technician and pathologist is permanent (8:30 to 17:30). A pathologist is on call beyond this period (U.Z. Gasthuisberg, Leuven)

Yes, although a dedicated TuBaFrost technician has not been appointed yet (CRO Aviano)

Yes (Oxford)

Difficulties encountered/foreseen?

The transfer after optimization of the protocols to routine technicians and pathologists will be very difficult due to the high workload of the routine pathology laboratory personnel (ERASMUS)

At the beginning, we introduced the TuBaFrost strategy for collecting tissue in a clinical session at which the surgery (dermatology, urology, and general) and the pathologist services were present. We explained the TuBaFrost project and emphasised the need to preserve fresh frozen tissues of high quality for use in research studies (Valencia Institute of Oncology)

None (U.Z. Gasthuisberg, Leuven)

None (CRO Aviano)

Additional points for consideration:

Good disposition of the surgery services for providing samples as indicated in the TuBaFrost SOP. (Valencia Institute of Oncology)

TuBaFrost recommendation: The specified time limit from excision of tissue to snap freezing is 30 minutes.

Key points: Minimise action of hypoxic phenomenon on gene expression and prevent tissue degradation. Of note, the Wales Cancer Bank and Chernobyl Tissue Bank adhere to a 15 minute time limit. In the event of the tissue not being snap frozen within 30 minutes, the lag time from excision to snap freezing must be recorded.

Has this been implemented in your institution?

The lag time is still too long. Times of 2 to 3 hours have been recorded. We are in the process of running a so-called "quality circle" on this subject, which comes to its final stage. The total success of this process is now depending on a budget requested, which is needed for fast transport. (ERASMUS)

Yes (Valencia Institute of Oncology)

All tissue is frozen within minutes (how long exactly, not recorded) after it reaches our facilities, but there is no control possible on the time-lag between excision and delivery at the pathology department. (U.Z. Gasthuisberg, Leuven)

Yes, this procedure was already implemented before the TuBaFrost protocol. (CRO Aviano)

Yes (Oxford)

Difficulties encountered/foreseen?

The large transport distance from the operating theatre to the department of pathology causes problems obtaining the tissue within the designated time limit. There is no form of fast transport, and only transport at certain times of the day. Due to the high number of fresh surgical resection specimens (also those of which it is not sure if it will lead to a frozen sample for the tissue bank) it is not cost efficient to let expensive personnel like a research technician take care of the transport other than in pilot protocols.

The operating theatres are a long walking distance from the Pathology department. It takes about 15-20 minutes to walk to and fro. Therefore the transport of resection specimen to the department of Pathology is taken care of by a messenger, which on settled times, brings the samples to the department. In addition, personnel of the operating theatre bring the fast diagnosis material for frozen sections to the department of Pathology. The lag time between taking out the tissue and freezing could in exceptional cases reach 3-4 hours. Since this is unacceptable for the material to be of use for experimental use, we started a so-called "quality circle" in our institute. The quality circle is an instrument to get all parties involved in a certain process that needs to be revised, around the table to discuss and solve the problems in the process. To start a quality circle you have to report the problem to the quality platform of your department and they will organize meetings with the personnel involved directly with the process.

The tissue bank had to prepare a short statement describing the problem in the process:

Through investigation we found that the time elapsing between the removal of tissue from the patient in the operating theatre and freezing for secondary experimental use, is on average 2 –3 hours. This results in hypoxic phenomena in the tissue and changes expression of several genes. Therefore the tissue is no longer suitable to use for a number of experiments. Upon enquiry this could also give problems for diagnostics (e.g. Molecular Pathology and certain enzyme determinations).

The Erasmus MC Tissue Bank has to deal with International standards as coordinator of the European human frozen tumor tissue bank for quality of tissue banking. The quality standards require that tissue is snap frozen within 30 minutes after removal from the patient. This also means that from the 9000 registered and stored samples only a few are suitable for exchange on an International level.

In the meetings personnel of operating theatres of different locations, logistics, quality platform, pathologists, histology and tissue bank were invited. During the first meeting the problem was again presented. Subsequently we discussed the situation we would like to have. The meetings after the first all dealt with how to achieve the situation we would like to have. It appeared that the activities and wishes of Pathology were not known and that tissue bank operators were not familiar with the protocols and proceeding of the operating theatre. To solve this problem several actions were undertaken. Tissue Bank personnel presented the tasks and achievements to operating theatre personnel. Pathology protocols were discussed and developed describing how resection specimen and biopsy material should be treated for transfer to pathology.

The head of the tissue bank together with a surgeon set up a pilot protocol. The pilot protocol enabled tissue bank personnel to look at three different parts of the time consuming process of tissue transfer: the operating room, the transport, and proceedings within the department of Pathology

The surgeon took the lead in showing where things could be done differently in the operating theatre, as he knows where the freedom of action is to be found. The Pathology form needed to accompany the tissue to the Pathology department was prepared before the operation procedure started. During the operation the surgeon explained that he only needed to think of transferring the tissue during the operation and would give the personnel in the operating room the instructions to prepare the tissue for transport, notify the tissue bank and later to give the instruction to bring it to the designated transfer point. From the transfer point, just outside the operating theatres tissue bank personnel transported the resection specimen to the department of Pathology, where a pathologist and assistant were required to assess what material can be spared for the tissue bank without harming the diagnosis. Using this pilot protocol, the lag time came down to just under 30 minutes, proving that it was feasible. In addition, it resulted in understanding the need of:

Personnel dedicated for the transport (carrier)

Clear protocols for transfer of tissue from the operating theatre to the carrier and from carrier to Pathology

Clear protocols for pathologists and assistants for reception of fresh tissue

A general protocol for all operating theatres

We now have a budget reserved for a carrier, which will also respond to fast diagnosis calls and fresh tissue, plus a general protocol for the operating theatres and clear protocols at the Pathology department on how to treat fresh tissues. At the time the carrier is hired the general protocol will be rolled out over all operating theatres and the pathology protocols made active.

We still need an evaluating meeting within a few months to assess the overall effect. (ERASMUS)

None (Valencia Institute of Oncology)

Yes: we suppose the tissue is brought immediately, but forcing the organisation of the operating theatre to do so is impossible. (U.Z. Gasthuisberg, Leuven)

Additional points for consideration:

Our Institution has provided the full time technician with a mobile telephone. Once the tissue has been removed from the patient, the surgery personnel phone the technician and he/she goes quickly to the surgery room and takes the tissue to the Department of Pathology. (Valencia Institute of Oncology)

TuBaFrost recommendation: Dissect the biopsy using aseptic technique

Key points: Use clean instruments for each resection and clean/change the instruments between dissecting normal and tumour tissue

Has this been implemented in your institution?

Yes, although not yet everyone has become accustomed to the cleaning of instruments between dissecting normal and tumour tissue. Clean instruments for new dissections are always used.
(ERASMUS)

Yes (Valencia Institute of Oncology)

Yes (U.Z. Gasthuisberg, Leuven)

Yes (CRO, Aviano)

Yes (Oxford)

Difficulties encountered/foreseen?

Cleaning/changing between dissecting normal and tumour tissue will be often forgotten
(ERASMUS)

None (Valencia Institute of Oncology)

None (U.Z. Gasthuisberg, Leuven)

None (CRO, Aviano)

TuBaFrost recommendation: The suggested size of tissue for snap freezing is approximately 0.5 cm².

Key points: The amount of tissue available will depend upon the sample site. Duplicate samples will be collected if there is sufficient material. Should the size or the weight be the specified criteria?
Avoid areas of necrosis.

Has this been implemented in your institution?

Sizes are varying and note is made of the approximate size: Small, Medium and Large, which stands for approximately <1 cm³, 1cm³ and >1cm³, respectively. (ERASMUS)

The size or weight should mainly be the specified criteria whenever it does not compromise the histopathological diagnosis (Valencia Institute of Oncology)

Yes (U.Z. Gasthuisberg, Leuven)

Yes (CRO, Aviano)

Yes (Oxford)

Difficulties encountered/foreseen?

I would rather see the 0.5 cm³ as a minimum, because this will take up more room in long term storage (ERASMUS)

With the size criteria, we are introducing a slant. Most of the tumours that will be introduced in the TuBaFrost database will correspond to highest stages (Valencia Institute of Oncology)

It is sometimes necessary to collect larger samples, due to difficulties when cutting (particularly in the case of lymph nodes) (CRO, Aviano)

Additional points for consideration:

Areas of necrosis are avoided, however in our quality control we have to adjust about 1% to necrotic samples. This was measured over 2003, whereas instruction in the staff led to a better score of only 0.1% so far this year. (ERASMUS)

Duplicates of samples not started yet (CRO, Aviano)

TuBaFrost recommendation: The tissue sample should be snap frozen in isopentane, either directly or embedded in cryosolidifiable medium.

Key points: Isopentane, in comparison to liquid nitrogen, causes less damage during freezing as it remains in a liquid state so there are fewer cryo-artefacts. This contrasts to the freeze-boil effect observed when using liquid nitrogen. Isopentane is a very good cryoconductor and allows rapid freezing.

Care should be taken during freezing to ensure the sample does not crack. Remove samples from isopentane and enclose in a cryovial or other storage vessel.

Has this been implemented in your institution?

Freezing the tissue samples is performed oriented, however not with OCT. The tissue sample is put on a piece of cork having approximately the size of the tissue to support and an equally sized piece of Whatman soaking paper soaked in physiologic salt solution. The site from which the sample for paraffin embedding is cut is directed upward, whereas this paraffin sample is oriented with the cut site upwards in the block. Since the quality of the frozen sample is not harmed in any way by this differing method we will continue the protocol in this way. (ERASMUS)

Yes (Valencia Institute of Oncology)

Yes (U.Z. Gasthuisberg, Leuven)

Not yet (CRO Aviano)

Yes - not with OCT (Oxford)

Difficulties encountered/foreseen?

When collecting of tissues will expand to peripheral hospitals, the availability of liquid nitrogen and isopentane could become a problem. (ERASMUS)

Difficulties in changing habits long settled in the Lab (CRO Aviano)

Additional points for consideration:

References for applicable papers:

TuBaFrost recommendation: Use a bar-code system for labelling the samples; this will result in improved samples management and precise identification. The bar-code should be used in conjunction with the TuBaFrost code 'TF_institution code_sequential code' so that the sample identifier is readable at institutions without bar-code scanners.

Key points: Use waterproof pen and labels 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 sample is coded-linked so that key individuals are able to access other relevant datasets.
If bar-codes are currently in use at your institution please provide more detail, e.g. number of characters/numbers.

Has this been implemented in your institution?

The storage vessel is labelled with the local code, whereas the TuBaFrost code will only mean that the TF institution code must be added. Since storage vials and numbering procedures differ per institute chances that two numbers in a set are equal and also in the same type of container are practically zero. Therefore, we do not want to renumber the vials upon issuing, but give the relation together with the minimal dataset sheet as is defined in WP6 and WP4. If a TF code is issued to our institute we can add this TF code to our local ID number.

The TuBaFrost Deliverable 3.1 and 3.2 give strong recommendation to a barcode system. At the moment our labelling procedure consists of a printed label, which is applied in the front of the vial. In addition, the lid and the bottom are marked with waterproof permanent pen. In the future, however, a Laboratory Management System will be installed at our department. From that moment on plans are to change the marking system to barcodes. The marking will be with 2D barcodes and text in front of the vial and a text/barcode only sticker on the lid, with stickers and durable ink guaranteed to hold in liquid nitrogen and that can withstand fast temperature changes. (ERASMUS)

YES, but not the bar-code procedure. (Valencia Institute of Oncology)

We use a sequential number, marked with a waterproof labelling. A prefix to the number indicates the location (and the type of tissue) of the sample. The use of bar-codes is not (yet) implemented (U.Z. Gasthuisberg, Leuven)

Not yet (CRO Aviano)

Not yet (Oxford)

Difficulties encountered/foreseen?

High prices of the printer and readers.

Non-compatibility of a 2D bar code. Considering using 1D instead if readable. (ERASMUS)

None (U.Z. Gasthuisberg, Leuven)

Difficulties in changing habits long settled in the Lab (CRO Aviano)

TuBaFrost recommendation: Record inventory details in a dedicated inventory book and in a password protected electronic inventory database (with varying levels of access).

Key points: Information recorded in inventory (at a minimum) – TuBaFrost code (plus bar-code if in use), location co-ordinates, pathology number, type of tissue and date of collection.
Record at this point if tissue is likely to be in any way infectious?
Database must be updated regularly when samples are moved or depleted.

Has this been implemented in your institution?

Yes (ERASMUS)

Yes (Valencia Institute of Oncology)

Yes, the TuBaFrost accessory number (or any other number) gives access to all data recorded - if permission is granted to the person logged in. (U.Z.Gasthuisberg, Leuven)

Yes, except for the TuBaFrost code (CRO Aviano)

Yes (Oxford)

Difficulties encountered/foreseen?

Logging the movements/depletion of the samples is not (yet) implemented (U.Z.Gasthuisberg, Leuven)

Additional points for consideration:

References for applicable papers:

TuBaFrost recommendation: Store the sample in an appropriately secure and maintained liquid nitrogen bank or -80°C freezer. Store duplicate samples independently if storage facilities are available.

Key points: Adequate maintenance – frost free, incident record book, temperature monitors, lockable repository.

Alarm system - local alarms, central alarms and dial-out system.

Contingency - repository of similar size and specification available for transfer of samples in the event of major breakdown, repository may be wired into hospital network (emergency generators).

Cryovials should be stored in the vapour phase of liquid nitrogen or sealed in Cryoflex to avoid explosions.

Has this been implemented in your institution?

So far, the Erasmus MC tissue bank did not have the availability of a back up system. Therefore we ordered a back up nitrogen storage facility hooked up to the central filling and alarm systems. Revision of the automated liquid nitrogen filling system appeared necessary to install this storage facility.

We have adequate maintenance through maintenance contracts of our storage equipment (liquid nitrogen), which is noted in log books. The liquid nitrogen level is high giving a longer time period for reaction after incidents occur without the risk of thawing the stored samples. Therefore we don't have temperature monitors.

The long-term repositories are locked and keys are kept by tissue bank personnel. All storage facilities are connected to the alarm system, leading to signals from a local alarm, central alarm and finally a dial-out system.

For contingency, one empty but operational storage barrel of similar size and specification is made available for transfer of samples in the event of major breakdown.

All storage facilities are equipped with a centralised and automated filling system.

Cryovials are stored under liquid nitrogen and all have a sealing ring in the lid to avoid explosions. (ERASMUS)

Yes (Valencia Institute of Oncology)

A duplicate storage is not implemented. The main laboratory is approx. 3km from the operating theatre. Alarm systems are implemented. The freezers are locked. (U.Z.Gasthuisberg)

Yes. Duplication of samples has not started yet (CRO Aviano)

Dial out alarm system instalment in progress, samples stored in vapour phase of liquid nitrogen, duplicate storage in place for breast tissue bank but not yet for lung and lymphoid tissue bank, storage repository now locked (Oxford)

Difficulties encountered/foreseen?

At the moment we have not duplicated samples in different storage facilities.

We preserve the tissues at -80°C. (Valencia Institute of Oncology)

None (U.Z.Gasthuisberg)

TuBaFrost recommendation: New institutions wishing to join TuBaFrost will be subject to an initial quality control accreditation. Quality assurance is fundamental to the successful operation of any repository that collects, processes, annotates, stored and distributes biospecimens for research purposes.

Key points: The accreditation will focus upon – freezers (security, capacity); computer hardware; documented technical protocols; and evidence of an informed consent document for tissue collection.

Additional points for consideration – staff training records

Do you think there should be further points considered for accreditation?

The documented technical protocols should meet the minimum standards set by the TuBaFrost Consortium (ERASMUS)

Personnel availability. Scientific interest of the implied pathologists. (Valencia Institute of Oncology)

No. The collection of the informed consent is done by the clinician, not the pathologist.

(U.Z.Gasthuisberg, Leuven)

No. What is listed is enough (CRO Aviano)

No (Oxford)

Difficulties encountered/foreseen?

Additional points for consideration:

Evidence of an informed consent document for tissue collection is only required when the local national law requires this procedure. Many countries have other forms, like consent or opt out. Opt out was accepted as the minimum? The question should read, that if consent procedures are needed due to local law and regulations, compliance to these rules and regulations should be clearly described in protocols.

References for applicable papers:

TuBaFrost recommendation: During Year 1 there should be a quality control review of 2% of new cases twice per year. If no problems are encountered this should be reduced in the second year to 1% of new cases reviewed annually.

Key points: The review will focus upon – Records and files (consent; minimal dataset; SNOMED coding; general request, incident and activity records), Equipment (technical maintenance) and the actual frozen sections.

Do you think this percentage of checks would be adequate?

Yes. (ERASMUS)

It would depend of the number of collected samples. I consider that if the number of samples is low the quality control review should not exceed more than once per year. What should be the minimum number of samples? For example, more than 200 samples during the first 6 months.

(Valencia Institute of Oncology)

We cannot organise an annual review of 2% of the cases. (U.Z.Gasthuisberg, Leuven)

Yes (CRO Aviano)

Yes (Oxford)

Difficulties encountered/foreseen?

Additional points for consideration:

References for applicable papers:

TuBaFrost recommendation: The quality control of the frozen samples will focus upon sample identification, review of stained H&E sections and quality of extracted RNA

Key points: Whilst checking the sample identification and location, the durability of the sample vessels and the inventory containers can also be checked to ensure they have remained stable at low temperatures. The stained H&E sections will be reviewed by a pathologist to confirm the diagnosis and assess how representative it is of the sample. The quality of the extracted RNA will be checked using an agarose gel or a bioanalyser - the bioanalyser requires less material and is quantitative.

Do you think there should be further points considered for quality control?

Due to the preparation of H&E slide of the tissue directly adjacent to top of the frozen sample an extra moment of Quality Control is introduced when a trained pathologist compares these produced slides to the diagnosis, before digital images are made. In addition, the images can be used during selection of the tissue for quality control.

The coming years the whole department of Pathology will be described in a quality handbook for certification of a Dutch hospital quality program called CCKL. The Tissue bank will be described in this handbook and will be part of the certification.

To implement the so far suggested quality control steps we have to introduce a yearly 2% check on new not rare samples for diagnosis, place in the system and stability by preparing RNA from the sample.

Generally no further points (ERASMUS)

No (Valencia Institute of Oncology)

No (U.Z. Gasthuisberg)

No, what is listed is enough (CRO Aviano)

No (Oxford)

Difficulties foreseen?

Additional points for consideration:

The stained H&E sections will be reviewed by a pathologist to confirm the diagnosis and assess how representative it is of the sample. This is standard for all our collected samples from the beginning of 2003. (ERASMUS)

Should the H&E sections be performed from the fresh-frozen tissue? What happen when the sample is in the cryovial without an embedded fluid (like OCT)? (Valencia Institute of Oncology)

Since the samples stored in the department using the same procedures (for diagnosis or research) give adequate quality of the RNA extracted, there is probably no use to spend frozen material for extra quality control. The frozen material is (can be) controlled further during the diagnostic workup, since the same material is used for immunohistochemical analysis. Frozen sections are post-fixed and stored with the paraffin sections. (U.Z. Gasthuisberg)

References for applicable papers:

TuBaFrost recommendation: Blocks and slides should be stored under appropriate conditions to prevent degradation and the inventory system should be secure and maintained.

Key points: Storage – climate controlled room (temperature and humidity) or in a refrigerator, controlled exposure to direct sunlight, frozen sections in a freezer.
Slide storage options - protective layer of paraffin, under vacuum, under gaseous nitrogen.
Inventory system – ordered filing system, light exposure controlled, lockable repository, controlled access.

Has this been implemented in your institution?

Blocks and slides are stored in special storage cabinets in a room in the centre of the building under the influence of the climate control and air conditioning of the whole building. The inventory is ordered under Pathology number, which is coupled to the hospital information system. Until recently the system had a provisional system for lending and issuing blocks and glass slides. This is now coupled to the LMS system with a bar code reading system. (ERASMUS)

Not at this moment (Valencia Institute of Oncology)

The blocks are not stored in a climate controlled room. Stained slides are coverslipped, and kept in ambient temperature. Unstained frozen sections are kept at minus 20°C. The inventory system is fully electronic and has a controlled access (U.Z. Gasthuisberg).

It's being implemented now (CRO Aviano)

Yes, frozen sections are kept at -80°C. Routine diagnostic blocks and slides are kept in a controlled access room. (Oxford)

Difficulties encountered/foreseen?

We do not have enough space (Valencia Institute of Oncology)

None (U.Z. Gasthuisberg).

Additional points for consideration:

We feel that the type and duration of the fixation is more important than the ambient temperature in the preservation of blocks for molecular analysis. (U.Z. Gasthuisberg)

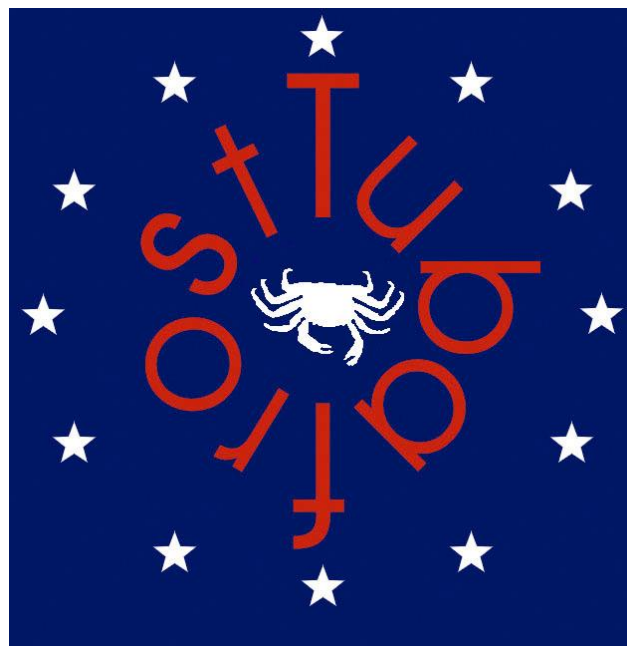
References for applicable papers:

Quality of Life and Management of Living Resources
**European Human Frozen Tumor
Tissue Bank**

TUBAFROST

QLRI-CT-2002-01551

Deliverable D 2.2
**Use of a basic prototype system at the participating
institutions**



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**TUBAFROST Work Package 2:
Development of storage system(s) for
frozen tissue (technical aspects).
(Deliverable 2.2 February 2004)**

This deliverable builds upon the selection of state of the art suitable tools to build a practical system for frozen tissue storage. More specifically this deliverable aims to evaluate the best practical procedure to store micro-biopsies for subsequent nucleic acids extraction.

Problems to consider

In Deliverable 3.2 the specifications for sample vessels are:

“Cryovials, cryomolds or other storage vessels (e.g. cryostraws) used for storing tissue for the TuBaFrost tissue bank must be:

- specifically designed for storing biological materials at temperatures as low as -190°C ;
- stable when submitted to sudden low temperatures (snap freezing), when held at low temperatures for long periods of time (years) or when taken through several freeze-thaw cycles; and
- as leak proof as possible (applicable to cryovials) even at the lowest cryogenic temperatures.”

The sample vessels used in the pathology departments and by TUBAFROST participants vary considerably in volume and shape. This lead to several problems:

- The volume of tissue stored in the sample vessels influences the tissue quality and eventually the quality of derived material (nucleic acids, proteins)
- This heterogeneity limits the development of automatized systems.

In many instance in oncology micro-samples are the only possibility to get access to tissue especially when organs are small, hardly accessible or when sequential samplings (for instance under treatment) are to be considered. Large sample vessels (for instance with a volume of 2ml or more) are inadequate to store these micro-samples. Moreover, cytological samples are being largely considered as fine needle aspirations could bring enough material to study the transcriptome, proteome or even to characterize immune response.

In Deliverable 3.2 the specifications for bar codes are:

- “The use of barcodes is recommended as it improves the accuracy of sample identification and can facilitate sample management and distribution. (...) the label and its adhesive must be able to a) withstand a wide range of temperatures (the minimum being approximately -196°C boiling point of liquid nitrogen) b) withstand an archival life of many years at extremely low temperatures and c) be self adhesive on many different materials;
- it is essential that the label remains firmly affixed and legible”.

However, it is difficult to fulfil these specifications without standardization of sample vessels and usually the 2ml cryovials does not allow secure adhesion.

In Deliverable 3.2 the specifications for storage of cryovials in liquid nitrogen begin by the following diagnosis:

“There is currently no screw top cryogenic vial on the market today that can claim to be leak proof in liquid nitrogen so the following recommendations are particularly important^{4,6}. If liquid nitrogen is trapped inside a container that is sealed, then expansion on warming above -196°C may cause an explosion, giving rise to danger from contamination by the vessel's contents as well as injury from fragments of the vessel itself.” Indeed, these “explosions” of the cryovials when warming up are a daily experience in most of the pathology departments.

Therefore, the selection of the best suitable tools to build a practical system for frozen tissue storage should handle these problems.

Qualitative and quantitative evaluation of extracted RNAs from micro-samples

Objective

To evaluate the possibility to obtain 3 µg of RNA in 90% of the cases from a sample taken with 18 Gauges and 14 Gauges needles with a 28S/18S *ratio* above 1.6. The RNA quality was evaluated using Agilent micro-chip.

Material

In 20 patients with breast carcinoma for whom a diagnostic fine needle biopsy have been performed, additional samples were obtained either with a 18G or 14G needle. In total, 46 samples were obtained for the study, distributed among breast (n=20), lever (n=9) and lymph node (n=17). For each site half of the samples were extracted with a 18G needle, the other half with a 14G needle. All the patients have signed a specific Patient Information Sheet/Informed Consent and the protocol was approved by the Institutional Review Board.

Methods

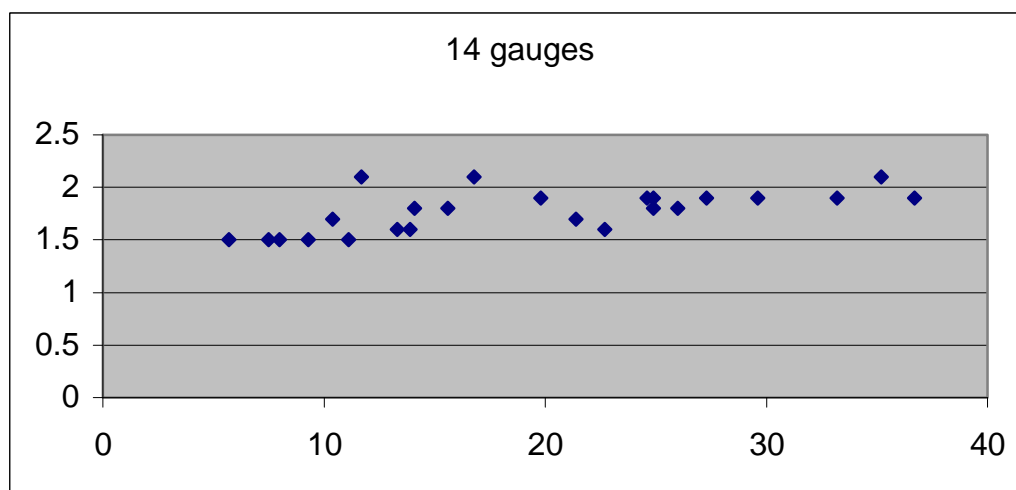
The sample was apposed against a glass slide for cytological evaluation, and then immediately put in a 2ml cryovial (Nunc cryovial) and the cryovial was directly immersed in liquid nitrogen. The samples were stored at least 7 days (range 7-25 days) in the standard nitrogen inventory container of the department. The samples extracted with a 14G needle were mechanically transformed in powder and then put in the lysis buffer (Qiagen) to follow the standard Qiagen procedure for double RNA/DNA extraction. The samples that were extracted with a 18G needle were directly added to the lysis buffer.

14G needles :

Average quantity in µg (range): 19.3 (5.7 – 36.7)

Average quality (range): 1,8 (1,5 – 2,1)

100% of the samples were above the target value (no technician-dependant effect)

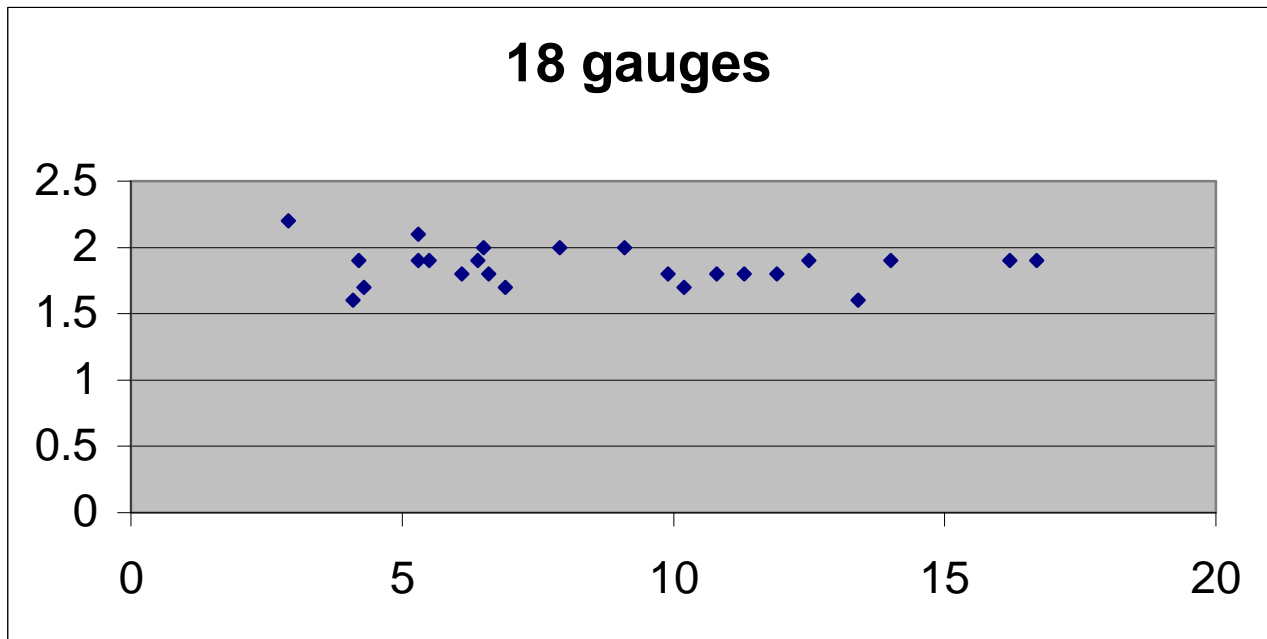


18G needles :

Average quantity in µg (range) = 8,7 (2,9 – 16,7)

Average quality (range) = 1,9 (1,6 – 2,2)

100% of the samples were above the target value but in breast samples, 3 profiles were partly degraded.



Results are therefore excellent in terms of RNA retrieval with both 18G and 14G needle aspiration. However it is extremely difficult to remove the sample from the 2ml cryovials that appear not appropriate for these samples.

Evaluation of straws in replacement of 2ml cryovials for sample storage in liquid nitrogen

We evaluated two different type of high-security straws developed by Cryo-Bio Systems™ for storage of biological samples in liquid nitrogen and nitrogen vapour. These straws have not been evaluated before for tissue storage as they were used only for liquids such as serum, plasma, buffy-coat and other blood fractions, cell suspension, bacterial or viral strains, gametes and embryos. These straws conform to [ISO 9002](#) standards.

Two different straws have been evaluated: 0.5 and 1.0ml that were pre-barecode-printed.

Their specifications are as following:

0.5 ml straws:

Length: 133 (+/- 1) mm before sealing ; 130 (+/- 1) mm after sealing

Diameter: 2.5 mm (internal) and 0.30 mm wall thickness

1.0 ml straws:

Length: 133 (+/- 1) mm before sealing ; 130 (+/- 1) mm after sealing

Diameter: 3.7 mm (internal) and 0.35 mm wall thickness



The three-part closure system guaranteed the biosafety of the sample during filling and emptying procedures. Airtight heat seals on both ends of the straw prevent contamination of both the samples and the environment. The cotton part that will allow to push the sample out of the straw.



Barcode-based tamper-proof identification system.
The straw has to be opened to remove the identification jacket.

Sampling

Several systems were tested: semi-automatized devices (14G Trucut™), 18G manual needles and 14G needles in several types of specimens received at the Department of Pathology of the Gustave-Roussy Institute. For the following types of specimens, between 3 and 17 samples have been made representing a total of 112 samples:

- Liver: free of lesion, colon adenocarcinoma metastasis, hepatocarcinoma
- Skin: squamous-cell carcinoma
- Lymph node: free of lesion, lymphoma
- Breast: free of lesion, adenocarcinoma
- Ovary: free of lesion, serous adenocarcinoma, mucinous adenocarcinoma, serous borderline tumour
- Soft tissue part: leiomyosarcoma
- Colon: mucosa, adenocarcinoma
- Thyroid: papillary carcinoma
- Pancreas: normal tissue, adenocarcinoma.

Results

For exophytic tumors of more than 2 cm in diameter the 3 systems were perfectly usable. They allowed to take a carrot of 0.8-1.2 cm in length. However for flat tissue (mucosa or skin) and non-exophytic tumors (for instance ulcerated colon adenocarcinoma), a carrot of tissue could have been taken only using a bistoury as used classically.

Sample introduction in the straw

Whatever was the sampling system only the straws of 1.0ml measuring 3.7mm in diameter gave enough space to introduce the needle and to leave the carrot of tissue in the straw in pinching it. Several systems were developed to try improving the process but no one appeared to be usable in routine. However, manually it was always possible to introduce the carrot in the middle of the 1.0ml straw without modifying its specifications.

Sealing

After the sample have been introduced into the straw, each extremity is thermically sealed using a specific device developed by CBS™.

Storage

Goblets are the removable storage elements placed in the liquid nitrogen container. Each goblet can hold sub compartments of different colors called visotubes. The straws are stored in a visotube. The full size central visotube protects the straw seal when goblets are stored on top of each other. Pre-assembled goblets with 12 visotubes were used. Each visotube could hold 9 straws of 1.0 ml, thus 108 straws fit in one goblet. The color composition can be adapted to the organ.

Goblets are stored on top of each other within each canister and a metal strip is designed for lifting up the goblets.

Storage and tissue use

The straws were stored in liquid nitrogen in a round storage repository. Thirty straws were randomly removed from the nitrogen and the carrot was removed out of the straw using a specific plastic baguette to push the cotton. Fifteen samples were weighted and used for histology control: after the whole freezing process, the tissue carrots weighted between 42 and 65mg. For all the specimens, after having been processed using a Cryostat at -20°C as for per-operative sections, the histology quality of the specimens was assessed by two observers and evaluated as "good" to "excellent", apart for a lymphoma that was "poor". The 15 other carrots were processed using the Qiagen™ procedure for RNA retrieval ("midi" columns). The RNA quality and quantity was in the same range than for 14G needles stored in 2ml cryovials.

In conclusion, the developed process allows to fulfil all the requirements described in deliverable 3.2. It facilitates the standardization of the sample volume which is a key factor in tissue repository.