available at www.sciencedirect.com

ScienceDirect

journal homepage: www.ejconline.com

TuBaFrost 2: Standardising tissue collection and quality control procedures for a European virtual frozen tissue bank network

M.M. Morente[a,*,m,n], R. Mager[b,c,m,n], S. Alonso[a,n], F. Pezzella[c,n], A. Spatz[d,n], K. Knox[b,n], D. Kerr[b,n], W.N.M. Dinjens[e,n], J.W. Oosterhuis[e,n], K.H. Lam[e,n], M.H.A. Oomen[e,n], B. van Damme[f,n], M. van de Vijver[g,n], H. van Boven[g,n], D. Kerjaschki[h,n], J. Pammer[h,n], J.A. Lopez-Guerrero[i,n], A. Llombart Bosch[i,n], A. Carbone[j,n,o], A. Gloghini[j,n], I. Teodorovic[k,n], M. Isabelle[k,n], A. Passioukov[k,n], S. Lejeune[k,n], P. Therasse[k,n], E.-B. van Veen[l,n], C. Ratcliffe[b,n], P.H.J. Riegman[e,n]

[a]Centro Nacional de Investigaciones Oncológicas (CNIO), Melchor Fernández Almagro, 3, E-28029 Madrid, Spain
[b]National Translational Cancer Research Network, University of Oxford, Radcliffe Infirmary, Woodstock Road, Oxford OX2 6HE, United Kingdom
[c]Nuffield Department of Clinical Laboratory Sciences, University of Oxford, John Radcliffe Hospital, Headington, Oxford OX3 9DU, United Kingdom
[d]Institut Gustave Roussy, Rue Camille Desmoulins 39, 94805 Villejuif, France
[e]Erasmus MC, Dr Molewaterplein 50, 3015 GE Rotterdam, The Netherlands
[f]U.Z-K.U-Leuven, Herestraat 49, B-3000 Leuven, Belgium
[g]Netherlands Cancer Institute, Plesmanlaan 121, NL-1066 CX Amsterdam , The Netherlands
[h]Alllgemeines Krankenhaus, University of Vienna, Waeringer Guertel 18-20, 1090 Vienna, Austria
[i]Fundación Instituto Valenciano de Oncología, C/Profesor Beltran Baguena, 8+11, Valencia, Spain
[j]Centro di Riferimento Oncologico, Via Pedemontana Occidentale, 12, I-33081 Aviano (PN), Italy
[k]EORTC Data Center, Avenue E. Mounier 83, B-1200 Brussels, Belgium
[l]Medlaw Consult, Postbus 11500, 2502 AM Den Haag, The Netherlands

ARTICLE INFO

Article history:
Received 22 March 2006
Accepted 4 April 2006
Available online 5 October 2006

Keywords:
Tumour tissue bank
Bio-repository
Tumour bank network
Standard procedures
Quality control

ABSTRACT

Tumour Bank Networking presents a great challenge for oncological research as in order to carry out large-scale, multi-centre studies with minimal intrinsic bias, each tumour bank in the network must have some fundamental similarities and be using the same standardised and validated procedures. The European Human Frozen Tumour Tissue Bank (TuBaFrost) has responded to this need by the promotion of an integrated platform of tumour banks in Europe. The operational framework for TuBaFrost has drawn upon the best practice of standard workflows and operating procedures employed by members of the TuBaFrost project and key initiatives worldwide.

* *Corresponding author:* Present address: Director, CNIO Tumour Bank Unit, Molecular Pathology Program, Spanish National Cancer Centre – CNIO, Melchor Fernández Almagro, 3, E-28029 Madrid, Spain. Tel.: +34 91 2246918; fax: + 34 91 2246923.
E-mail address: mmorente@cnio.es (M.M. Morente).
[m]M. Morente and R. Mager contributed equally to the article.
[n] The TuBaFrost Consortium.
[o] Present address: Istituto Nazionale Tumori, Via Venezian 1, 20133 Milano, Italy.
0959-8049/$ - see front matter
doi:10.1016/j.ejca.2006.04.029

1. Introduction

Provision of high quality human neoplastic and normal tissue annotated with clinical information is pivotal to the drug development process and translational research. In particular, the ongoing development of new molecular techniques has enabled large-scale studies of gene expression to be carried out that have a high clinical significance.

Tumour bank excellence is based on validated procedures for the collection, storage, retrieval, shipping and tracking of human tissue samples of clinical origin. Tissue bank networks offer an opportunity to store large numbers of samples at distributed sites, building on existing experience and infrastructure. Reproducible results from samples stored across such a network rely on standardisation of the methods of tissue collection, storage and processing in order to minimise the intrinsic biases of multi-centre studies. Much of the variation noted in studies that show conflicting results in the correlation of molecular markers can be explained by lack of standardisation of tissue collection and processing.[1] The TuBaFrost Project has responded to this need for high quality uniform samples through the development and promotion of an integrated platform of tissue banks in Europe.

The TuBaFrost model is not that of a Central Tumour Bank, but of a virtual bank, characterised by a central database and de-centralised sample storage (tissue is stored at the institute where it is collected), thereby playing a key role in the development of local molecular diagnosis, teaching and research activities. Simultaneously, it represents a tool for the promotion of multi-centre cancer research and for collaboration at the interface between different biomedical disciplines, for example between basic and clinical researchers. To ensure uniform tissue quality and in order for the outcome of different scientific experiments to be compared, standardised protocols for collection, storage, retrieval and tracking of tissues have been developed and implemented in all TuBaFrost collecting institutions. The operational framework for TuBaFrost has drawn upon the best practice of standard workflows and operating procedures employed by members of the TuBaFrost project and key tissue bank initiatives worldwide.

2. Strategy

The Standard Operating Procedures (SOPs) and quality assurance (QA) measures developed for TuBaFrost do not aim to create total uniformity in collecting, freezing, storage retrieval and tracking of the samples. Such uniformity would indeed equalise the quality of the samples, but would also lead to the implementation of measures which are not completely necessary for ensuring the quality of tissue samples. The cost of implementing such measures could deter institutions from becoming collectors. Instead the work flow for collecting and storing tissues was analysed (see Fig. 1) and the actions essential for producing high quality samples were described. Less essential actions were described as recommendations. This strategy resulted in compulsory measures and advisory recommendations, thus striking a balance between ensuring high quality samples and low

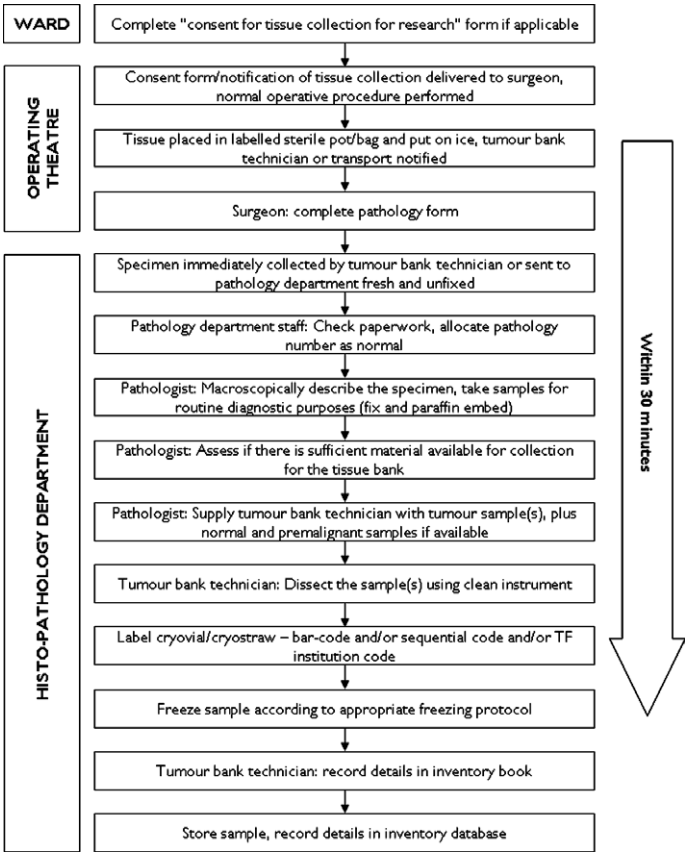

Fig. 1 – Model work-flow for the collection and storage of remnant human tissues within the tissue bank setting.

inter-institute variablity but also accessibility for new collecting institutions.

3. Organisational structure

Establishing an organisational structure for collecting remnant human tissues for research or education should minimise the likelihood that surgical specimens are compromised diagnostically.[2] It will also benefit the pathology department as specimens will be transferred from the operating theatre to the pathology department more efficiently, and the presence of a fully trained technician could help to address the shortage of pathologists and pathology department time available for supporting tissue banking activity. Integrating the tissue banking activities into the routine surgical and pathology activities is essential for the efficient acquisition of tissue[3] and it is vital that there is support for the tissue banking activities from the surgical and pathology teams. The close cooperation of the surgeon with the molecular biologist and drug development team could deliver a paradigm shift in clinical evaluation of novel therapies.[1]

From this tissue bank perspective, the main goal of establishing an organisational structure is to minimise warm ischaemia time and tissue degradation, which is essential in ensuring that the tissue is fit to be used for a variety of research purposes. Research by Huang *et al.* [4] states that the tissue should be frozen within 20 min, that this is the desired standard for human tissue preservation prior to cDNA or oligonucleotide microarray analysis and that the surgeon has the capacity to streamline standard operating procedures to minimise warm ischaemia.

4. Operating procedures

4.1. *Lag time and conditions between excision and freezing of tissue*

Reducing the lag time from excision of tissue to freezing is linked to the establishment of an organisational structure. The surgical specimens must be collected in the operating theatre immediately after their removal and transported in an unfixed state to the Pathology department. The best method for transferring the specimens is not to immerse them in liquids of any kind, but simply to place them in a closed container and to transport them with the same rapidity as for a frozen section diagnosis. The tissue should ideally be kept cool after excision by transporting it in a plastic container/bag on ice to the pathology department, this may delay degradative processes. Grizzle *et al.* [2] 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.

Post-resection variables, such as the temperature at which the sample is maintained between excision and snap freezing or the time lag between excision and freezing, can be controlled to a certain extent by the pathologist and tissue bank personnel. However, pre-resection variables, such as the time under ischaemic conditions at 37 °C during surgery, cannot be accurately predicted and controlled. Almeida *et al.* [5] compared changes in mRNA expression levels and RNA stability in fragments of mouse liver tissue exposed to 25 and 37 °C over 4 h and demonstrated that at 25 °C RNA degradation was limited, while at 37 °C both the 18S and 28S rRNA species were almost completely degraded. However, when changes in gene expression were assessed for seven genes through relative quantification of mRNA, only for one gene a reliable estimate of in vivo mRNA levels could not be made, even if performed on degraded RNA.

Snap freezing the tissue must be done as soon as possible after excision to ensure that there is minimal degradation of the specimen and hence no limitation on the types of studies that can be conducted nor any influence on the scientific usefulness of the data obtained from tissue analysis. According to Spruessel *et al.*,[6] control of variables such as tissue ischaemia time is mandatory to obtain reliable data in screening programs for molecular targets and diagnostic molecular patterns. Their study focussed upon the impact of ischaemia on gene and protein expression profiles of healthy and malignant colon tissue and revealed that initial changes in gene and protein expression profiles were already observed 5–8 min after colon resection. Fifteen minutes after surgery, 10–15% of all detectable genes and proteins differed significantly from baseline values, and 30 min after surgery this had increased to 20%.

Research by Huang *et al.*,[4] also on human colon cancer specimens, quantified the effects of warm ischaemia – aliquots of tissue were frozen at 5, 10, 15, 20, 40 and 60 min after excision and the microarray data analysed. They concluded that ischaemic times of less than 20 min provide relatively stable gene expression profiles, and that an ischaemic time of more than 40 min results in significant deviations from baseline.

A time-course study on differential gene expression of prostate tissue by Dash *et al.*[7] focussed on the fact that genes appearing to be up- or downregulated could represent an artefact of RNA degradation due to prolonged warm ischaemia time. Experimental data showed that less than 0.6% of the more than 9000 genes tested were affected by an ischaemic time of 1 h at room temperature. However, they concluded that some prostate cancer genes might be more susceptible to ischaemia, and all attempts should be made to process tissue rapidly to ensure that the microarray gene profile accurately reflects the state of the cells.

Consistent with this is a study by Blackhall *et al.* [8] focussing on the stability and heterogeneity of expression profiles in lung cancer specimens kept at room temperature and frozen at specific time intervals (from 5 to 120 min) after resection. Evaluation of the RNA extracted from these tissues showed no clear pattern of change in relative gene expression with time but it was concluded that it is prudent to snap freeze tumour samples as soon as possible after resection and ideally within 30–60 min from resection.

Based on the data from the various studies mentioned above, the lag time from excision to snap freezing recommended for the TuBaFrost project is 30 min (Fig. 1); it is however recognised that this may be impractical 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. Hence, tissue subject

to a delay in time from excision to snap freezing of up to 2 h should still be collected for the bank and the delay noted within the local database so that researchers receiving the samples are fully informed about the quality status. In general, the system established for collecting and transporting tissues for research should be developed around the specific operating policies of the institution.[2] However, when participating in a tissue bank network, minimum standards must be met to ensure that the experimental results can be legitimately compared.

4.2. *Dissecting the biopsy using aseptic technique*

It is recommended that clean instruments are used for each resection, and that they are cleaned or changed in between dissecting normal and tumour tissues. Clearly displayed protocols will be necessary to ensure that TuBaFrost procedures are embedded in routine pathology department practice of the collection centres. The use of sterile foil and instruments is recommended if the tissue is to be eventually used for RNA profiling.

4.3. *Tissue sample size and sample vessel*

With modern day tissue-sparing surgical techniques and in instances when target organs/sites are small, difficult to access, or when sequential samplings (for instance during treatment) are to be considered, residual tissue fragments available for banking are often very small (micro-biopsies). Ideally tissue collected for the TuBaFrost project will be approximately 0.5 cm^3 and if sufficient material is available it is recommended that more samples are collected, ensuring of course that the diagnosis is never compromised. This sample size is consistent with best practice throughout the TuBaFrost consortium, in other tissue bank initiatives, for example the Wales Cancer Bank and the Cooperative Human Tissue Network, and is also the selected size for some studies.[9] Frequently the main problem is not the sample size, but the tissue quality in terms of how representative the sample is histologically and also the absence of areas of necrosis.

There is no universal vessel suitable for each and every sample and each and every tissue repository, since the vessel directly depends on the type and volume of the sample and also the inventory system already in use. However, all vessels used for storing tissues for the TuBaFrost tissue bank must meet some basic criteria: they must be specifically designed for storing biological material at temperatures as low as −190 °C; be stable when submitted to sudden low temperatures (snap freezing) and when held at low temperatures for long periods of time (years); and be as leak proof as possible, even at the lowest cryogenic temperatures. Therefore, selection of the most suitable tools to build a practical system for frozen tissue storage should address these criteria.

When procedures and methods at all the participating TuBaFrost institutes were surveyed, it was unsurprising to discover that sample vessels varied considerably in volume and shape, from cryovials to cryomolds to cryostraws. This varying volume may influence tissue quality and also limit the development of standardised and potentially automated inventory systems across the network. However, in comparison to other factors, such as warm ischaemia time, the type of sample vessel is less important for sample quality and this variability was accepted and accounted for within the developed TuBaFrost SOPs.

Solid fragments from large or medium sized samples can be frozen in cryomolds embedded in a cryoprotector substance, OCT compound or similar. This type of storage vessel and protectant gives high quality results for histopathological study, including microdissection and tissue microarrays of frozen tissues. Published observations[10] have shown that the use of OCT is not suitable when the tissue is to be used for subsequent molecular biological analyses and can be unsuitable for small samples when used with certain vessels [11] – however, this method of cryopreservation preserves histology, protects the tissue from the freeze-burn effect of liquid nitrogen and minimises biological contamination of tissues, so it remains as an option for the collection of tissue for the TuBaFrost network.

Cryovials provide a functional and space efficient option for small or large tissue fragments and also cytological samples. Local Health and Safety regulations must be particularly adhered to when using cryovials as if liquid nitrogen is trapped inside the sealed cyrovial and expands on warming it 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.

To ensure that the TuBaFrost network advocates the use of the most appropriate practical procedure for storing these micro-biopsies for subsequent molecular analyses, a cryo-straw system[12] developed by Cryo-Bio Systems™ was evaluated. These high-security straws are designed for storage of biological samples in liquid nitrogen and nitrogen vapour and when trialled for tissue storage this system proved effective for fine needle biopsies, preserving histology, giving high yields of quality RNA and notably allowing for standardisation of the sample volume. This system fulfilled the storage vessel criteria for the TuBaFrost network and is included as an option within the TuBaFrost SOPs.

TuBaFrost SOPs will be periodically reviewed to ensure that they reflect current best practice and recent developments in tissue banking methodology, for example RNALater appears useful for decentralised sample collection coupled with high throughput gene analysis in a central location,[13] but can limit use of the tissue for other techniques. In addition, the development of novel methods of freezing samples such as the 'capsule-freeze' method [11] could improve tissue preservation.

4.4. *Freezing procedures*

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 efficient cryoconductor, allows rapid freezing and 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 with 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 can be frozen in liquid nitrogen.

To pre-cool the isopentane, the vessel 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 that the sample does not crack.

Although the pre-cooled isopentane method seems to be the most suitable one, it is not free of disadvantages as it is time-consuming, complex, and poses an additional biohazard. Some TuBaFrost participating centres prefer the use of pre-cooled isopentane at −80 °C kept in mechanical freezers, or using specifically designed equipment at −55 °C (*e.g.* Histobath©).

4.5. *Sample labelling*

In each collecting institute's local tissue bank, the samples are generally identified by a local inventory code or bar-code – this should in no way relate to the pathology number or other patient identifiers. Inventory details must be recorded in a dedicated inventory book and in a password-protected electronic inventory database, with varying levels of access. The information recorded in the inventory should at a minimum include the data referred to in the TuBaFrost central database.[14] The samples should be coded-linked so that key individuals with appropriate access rights can interrogate other relevant datasets, *e.g.* clinical data, follow-up data. The local inventory code and bar-code can be used in conjunction with the TuBaFrost code 'TF_institution code_local inventory code' or not. However, when a sample is issued to a requestor, it must be annotated with the TuBaFrost code in order to ensure tissue traceability, and be accompanied by relevant documentation.

Use of a bar-code system for labelling the samples will result in improved sample management and precise identification. In the absence of a bar-code system, a waterproof pen and labels able to withstand storage at ultra-low temperatures should be used.

4.6. *Tissue storage*

Effective and secure long-term storage of tissue samples is essential for the TuBaFrost network, the samples must be stored in an appropriately secure and maintained liquid nitrogen bank or −80 °C freezer. 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. This is consistent with suggestions [15] that temperatures at 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. Evidence from Chu *et al.*[9] shows that in general the integrity and yield of DNA from gynaecological tissues remains unchanged with long-term freezing, whereas the integrity of RNA becomes compromised after storage of 5 years or longer but amplification of short fragments by reverse-transcription polymerase chain reaction (RT-PCR) is still successful. Experience[16] 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 for storage at Biopathology Centers, OH, USA.

Contamination issues regarding the use of liquid nitrogen and detailed by Burden[17] can be avoided by using liquid nitrogen in the vapour phase – this can also avoid the potential for cryovial explosions described earlier.

On the other hand, and mainly for small and medium institutions, the storage of frozen tissue in chest or upright freezers at −80 °C has some advantages in comparison to liquid nitrogen storage, such as greater sample accessibility, simpler installation, fewer maintenance requirements, and typically more economical to purchase and maintain. However, liquid nitrogen does guarantee a lower and more stable temperature compared to variations in temperature experienced in mechanical freezers [18,19] though there still temperature gradients experienced when using liquid nitrogen in vapour phase.[17]

As mechanical freezers depend on the electrical power supply network, they will require appropriate security measures to minimise the risk of large temperature fluctuations or complete failure. It is advised that the mechanical freezers are incorporated into a secure electricity supply if available, in such a way that if the power fails emergency generators will ensure continuity of supply. In addition, it is recommended that for both mechanical freezers and liquid nitrogen repositories a triple-layer alarm system is employed which will monitor any temperature increases of more than 10% above −80 °C. This incident will trigger a progressive series of alarms:

- Local alarm (acoustic and visual), in the room where the repository is located and in a nearby room.
- Distant alarm (acoustic and visual). If after a set period of time the local alarm has not been attended to, an alarm will be activated in a distant room in the same building or complex which is manned by maintenance staff 24 h a day. This alarm will not be effective unless clear and concise written instructions are left indicating what protocol should be followed; and
- In the absence of either local or distant alarms being effective or if there is no maintenance staff station, a dial-out system should be put in place with telephone localisation to contact a series of pre-programmed numbers. It is recommended that a contingency plan is developed and clearly displayed close to the repository, in particular detailing the location of other freezers of similar dimensions and characteristics into which the tissue may be transferred in the event of breakdown or when the main equipment is being cleaned.

Dual storage of samples in geographically separate storage facilities and the potential security advantages for repositories was discussed by the TuBaFrost consortium. It was felt that if adequate risk management and security measures are in place then duplicate storage is not essential for the collecting institutes of the TuBaFrost network.

4.7. *Block and slides storage*

Blocks and slides produced for routine diagnostic purposes or slides produced from frozen sections are of great value as a complement to the frozen repository. They should be stored under appropriate conditions to prevent degradation and coupled with a secure (lockable and controlled) access, accurate and up-to-date inventory system. Paraffin embedded tissues and slides should be stored under climate controlled conditions (temperature, humidity and sunlight). Frozen sections must be stored in a freezer.

4.8. *Biohazards*

All handling of human tissue carries a risk of exposure to infectious agents that, although it cannot be completely eliminated, must be minimised as far as possible. It is currently impossible to fully ensure the absence of high-risk agents through specific tests of all patients and samples – in fact, these data are not always recorded in the clinical history of the patient. Therefore, researchers are advised to treat all samples as if they are contaminated.

Training programmes for tissue bank personnel must focus on safety issues as well as on 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.[2]

Working with freezers, liquid nitrogen and isopentane is hazardous, and therefore all procedures should comply with local safety rules specific for these chemicals and equipments.

5. Quality assurance policy

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 and, in the case of a network, in the Central Office, especially when collecting and distributing tissues from many different institutes.

It is necessary to highlight the differences between quality assurance (QA) and quality controls (QC). Quality assurance refers to a general, documented and ideally certified policy of quality. Quality controls are a very important part of the quality assurance but they are only a mechanism to evaluate and improve the quality of the system.

A valid QA policy must include

- 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 allow material in appropriate conditions to be delivered, and some form of validation must be performed to assure acceptable reliability/reproducibility;
- a process map for the work;
- Standard Operating Procedures (SOPs);
- use of traceable reference materials to enhance standardisation of the quality of processing;
- other documents and forms recorded within the system;
- document control (archiving, review, amendments, storage, *etc.*);
- documentation and review of staff training and competence; and
- auditing procedures to maintain standards and to normalise processes performed in different centres.

An initial accreditation should focus on the infrastructure: freezers, computer hardware, documented technical protocols, evidence of an informed consent document for tissue collection (if applicable in country of collection) and staff training records.

Adequate information and training is essential in a large tissue bank network: staff of the Cooperative Human Tissue Network, USA,[20] which consists of six divisions and distributes approximately 80,000 research specimens to researchers annually, undergo annual training at the Biopathology Centers (BPC), Ohio, with particular focus on timely snap-freezing of tissue.[16] Feedback from researchers on the quality of RNA and DNA supplied allows annual verification of institutional performance.

The model for the TuBaFrost quality assurance policy creates two systems according to the length of participation of the collecting centre and the centre's results record. In the first year of collection, an institute will 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, the quality review will be reduced in subsequent years to 1% of new cases received annually. The regularity of review will depend on the number of tissues banked; for example, if there are more than 2000 samples collected during the first 6 months, the review will take place twice a year.

The review will focus on records, equipment, frozen sections and fixed sections – as detailed in Table 1. Stained H&E frozen tissue sections will be reviewed by a pathologist to confirm the diagnosis and to assess how representative the sample is. RNA will be extracted and the quality checked using an agarose gel or a bioanalyser – the bioanalyser requires less material and provides integrity and concentration information. Sample records will also be checked for accuracy and physical checks of the sample identification and location will also allow the durability of the sample vessels and the inventory containers to be assessed to ensure that they have remained stable at low temperatures.

Fixed sections will not routinely be collected for TuBaFrost, but may be useful to provide an additional level of quality control and prevent wasting frozen tissues. The quality of the paraffin embedded tissue can be checked by immunohistochemical staining (Vimentin,[21], Ki67, CD34 and/or others) to evaluate optimal sample fixation (antigen preservation) in paraffin blocks. For this purpose the use of tissue-arrays is recommended, allowing testing of antibodies in the same conditions with a limited number of slides, thus minimising the final cost and the number of slides to be sent to the associated centres for in-house checking.

Table 1 – TuBaFrost quality control

In each hospital/centre
Review of 2% of new cases, twice a year during first year
◦ Cases are selected at random but only from the common cases and those with sufficient tissue
◦ 2% review twice a year in the first year a hospital/centre belongs to the Network. If no quality problems emerge, then this can be reduced to 1% or similar
Records and files:
◦ Appropriate informed consent (appropriate to the country of origin)
◦ 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, sex localisation, age
◦ Appropriate SNOMED / CIE-O 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 representativeness 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)
Frozen tissues
◦ Reviews of stained HE sections by a pathologist in order to assess/ confirm the diagnosis and how representative they are of the samples
◦ Review of the sample identification (bar code, *etc.*)
◦ RNA extraction and quality assessment
In the central office
About records and files:
◦ Appropriate SNOMED codification (if centralised)
◦ Registries of activity

This quality system operates successfully for the distributed network of the CNIO Tumour Bank Network in Madrid.[22] Establishing and adhering to a quality system may present an initial challenge for some institutions, but with clear guidelines supplied by the TuBaFrost network it can only improve the quality of all the tissues collected and increase the potential collaborations for the institute.

6. Concluding remarks

Technical developments have renewed the need for high-quality tissue samples, especially since this technology can yield data of increasing clinical value. To identify new parameters of clinical importance, we are obliged to run large-scale molecular studies with large numbers of cases, and treatment response prediction must be based on clinical trials, which include the acquisition of clinical samples for molecular studies. The transfer from basic to clinical research requires homogeneous tissue-sampling protocols that allow multicentre studies.

In this context, Tumour Bank Networking presents a great challenge for oncological research,[23,24] and must be based on cooperative designs and on standardised, suitable and available procedures in such a way as to make it possible to carry out molecular studies that avoid the intrinsic bias of multicentre studies.

Conflict of interest statement

None declared.

Acknowledgements

The European human frozen tumour tissue bank or TuBaFrost project was funded by the European Commission within the 5th framework of the division 'Quality of life and living resources' under project number QLRI-CT-2002-01551, with the aid and commitment of the involved scientific officers J. Namorado, M. Vidal, O. Kelm and S. Jungblud. The authors greatly appreciate the many collaborators (pathologists, technicians, health documentation experts, *etc.*) involved in the Tumour Banks of the TuBaFrost institutions and K.M.M. de Wildt for her management support during the project.

REFERENCES

1. Knox K, Kerr DJ. Establishing a national tissue bank for surgically harvested cancer tissue. *Br J Surg* 2004;**91**:134–6.
2. Grizzle WE, Aamodt R, Clausen K, LiVolsi V, Pretlow TG, Qualman S. Providing human tissues for research: how to establish a program. *Arch Pathol Lab Med* 1998;**122**:1065–76.
3. Naber SP. Continuing role of a frozen-tissue bank in molecular pathology. *Diag Mol Pathol* 1996;**5**:253–9.
4. Huang J, Qi R, Quackenbush J, Dauway E, Lazaridis E, Yeatman T. Effects of ischemia on gene expression. *J Surg Res.* 2001;**99**:222–7.
5. Almeida A, Thiery JP, Magdelénat H, Radvanyi F. Gene expression analysis by real-time reverse transcription polymerase chain reaction: influence of tissue handling. *Anal Biochem* 2004;**328**:101–8.

6. Spruessel A, Steimann G, Jung M, Lee SA, Carr T, Fentz A, et al. Tissue ischemia time affects gene and protein expression patterns within minutes following surgical tumour excision. *Biotechniques* 2004;**36**:1030–7.
7. Dash A, Maine IP, Varambally S, Shen R, Chinnaiyan AM, Rubin MA. Changes in differential gene expression because of warm ischemia time of radical prostatectomy specimens. *Am J Pathol* 2002;**161**:1743–8.
8. Blackhall FH, Pintilie M, Wigle DA, Jurisica I, Liu N, Radulovich N, et al. Stability and heterogeneity of expression profiles in lung cancer specimens harvested following surgical resection. *Neoplasia* 2004;**6**:761–7.
9. Chu TY, Hwang KS, Yu MH, Lee HS, Lai HC, Liu JY. A research-based tumor tissue bank of gynecologic oncology: characteristics of nucleic acids extracted from normal and tumor tissues from different sites. *Int J Gynecol Cancer* 2002;**12**:171–6.
10. Turbett GR, Sellner L. The use of optimal cutting temperature compound can inhibit amplification by polymerase chain reaction. *Diag Mol Pathol* 1997;**6**:298–303.
11. Loken SD, Demetrik DJ. A novel method for freezing and storing research tissue bank specimens. *Human Pathol* 2005;**36**:977–80.
12. Cryo Bio Systems. Available from: http://www.cryobiosystem-imv.com/.
13. Mutter GL, Zahrieh D, Liu C, Neuberg D, Finkelstein D, Baker HE, et al. Comparison of frozen and RNALater solid tissue storage methods for use in RNA expression microarrays. *BMC Genomics* 2004;**5**:88.
14. Isabelle M, Riegman PHJ, Morente MM, et al. TuBaFrost 5: multifunctional central database application for a European Tumor Bank. Eur J Cancer, Submitted simultaneously to complete the TuBaFrost series in EJC.
15. Karlsson JOM, Toner M. Long-term storage of tissues by cryopreservation: critical issues. *Biomaterials* 1996;**17**:243–56.
16. Qualman SJ, France M, Grizzle WE, LiVolsi VA, Moskaluk CA, Ramirez NC, et al. Establishing a tumour bank: banking, informatics and ethics. *Br J Cancer* 2004;**90**:1115–9.
17. Burden DW. Issues in contamination and temperature variation in the cryopreservation of animal cells and tissues Revco Technologies. *Application Note* 1999;**8**:99–108.
18. Helsing KJ, Hoffman SC, Comstocka GW. Temperature variations in chest-type mechanical freezers. *Clin Chem* 2000;**46**:1861.
19. Su SC, Garbers S, Rieper TD, Toniolo P. Temperature variation in upright mechanical freezers. *Cancer Epidemiol Biomarkers Prev* 1996;**5**:139–40.
20. National Cancer Institute Cooperative Human Tissue Network (CHTN). Available from: http://www.chtn.ims.nci.nih.gov.
21. Assessment of antigen damage in immunohistochemistry. The vimentin internal control. *Am J Clin Pathol* 1991;**96**:669–71.
22. Centro Nacional de Investigaciones Oncológicas, CNIO: http://www.cnio.es.
23. Morente MM. Tumour banks and oncological research. *Rev Oncol* 2003;**5**:63–4.
24. Morente MM, Alonso S. Current challenges of human tumour banking. *Hematol Oncol* 2005;**23**:54–6.