Quality of Life and Management of Living Resources

European Human Frozen Tumour Tissue Bank

TUBAFROST

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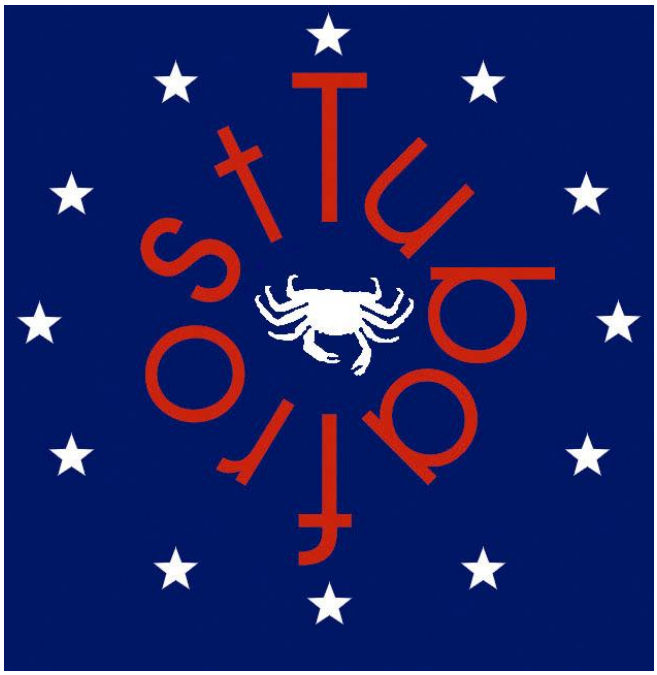
Deliverable D 3.2

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



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 throughout[4].

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 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 hazards[5]:

- 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

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 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 CO_2 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 repositories[4] 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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