Quality of Life and Management of Living Resources

European Human Frozen Tumour Tissue Bank

TUBAFROST

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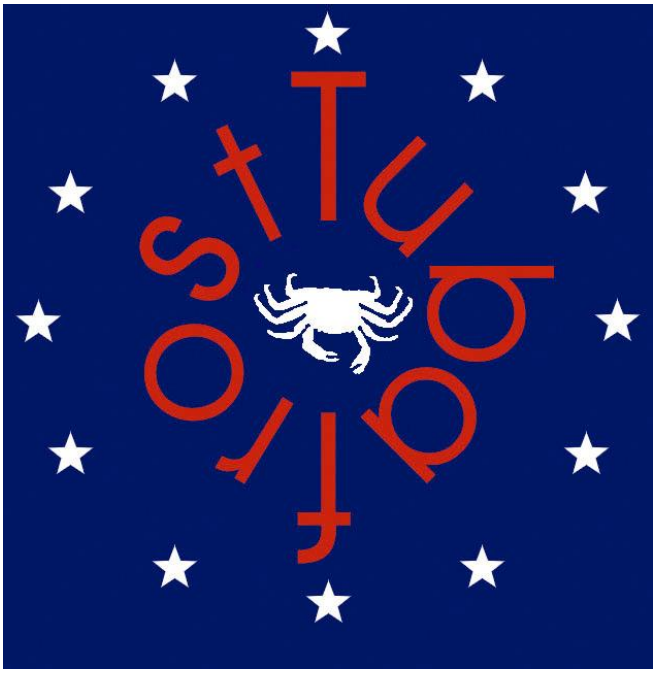
Deliverable 3.3

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



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 $cm^{2;}$
- 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; and
- 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 then 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 receipt 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 cm^2.

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 cm^3, 1cm^3 and >1cm^3, 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 cm^3 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:

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 -80ºC. (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: