

Annual report 2004 - WP 5.1 Pilot Tissue Biobanking

Johan Botling, MD PhD
Department of Genetics and Pathology
Rudbeck Laboratory
Uppsala University Hospital

Summary

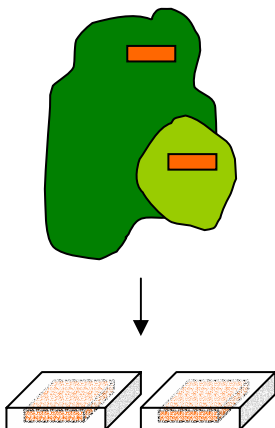
The aim of WP 5.1 is to optimise biobanking of non-fixed tissues. We have developed routines that enhance biobanking of fresh frozen tissue from surgical specimens and biopsies. A pilot biobank has been established where tissues embedded in cryogel are frozen and stored in blocks at -80°C. Cryosection and histopathological examination of each frozen case significantly improve characterisation of the biobank samples and ensures clinical diagnostic security. The important endpoint is tissue with preserved morphology and intact DNA, RNA and proteins. To this end we have implemented quality test protocols for DNA- and RNA-quality that can be performed on minute amounts of tissue. Logistics and biobanking routines have been adapted accordingly. We deliver samples to a large number of research projects aimed at tissue profiling by use of genomics, proteomics and gene expression analysis and serve as a core facility for tissue biobanking in Uppsala.

Deliverables

(1) Optimisation and evaluation of novel system for biobanking of non-fixed tissues /

(2) Tissue selection - Pathology

The logistics of the pilot biobank and the tissue selection have been described in detail in the annual report of 2002. In summary:



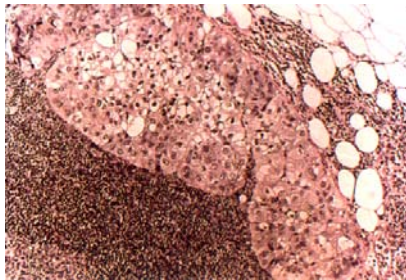
Non-fixed tissue samples are sent on ice (at 0°C) to the Department of Pathology.

Normal and lesional tissue (usually tumor tissue) is selected, covered with cryogel (OCT) in a cryomold, and snap frozen in isopentane/dry ice. Remaining tissue is formalin fixed for subsequent routine diagnostics.



High quality frozen sections are made from each frozen case (CryoJane Tape Transfer System) and HE-stained slides are included in the routine diagnostics along with the paraffin slides from the fixated fraction of the case.

A digital image of the frozen sections representing the biobank material is linked to the case data in the biobank database.



Database (Filemaker Pro Archive)
 biobank number
 case number
 "person nr"
 diagnosis
 SNOMED code (T/M)
 position in freezer
 usage in projects
 comments...

In 2004 this biobank concept was fully implemented at the Department of Pathology and all non-fixed tissue referred to us from Uppsala University Hospital is handled as outlined above. The pathologist and lab.technician on call for clinical frozen section diagnostics are responsible for the tissue selection. New manuals for handling colon-, breast-, brain surgery-, prostate- and bladder specimens have been implemented during the last year. Transport logistics was modified to some extent as a result of experiments testing RNA-quality (see below). The pilot biobank has boosted the net-growth of the frozen tissue biobank. This is due to an increased number of specimens being sent to us in a non-fixed state. Also, a higher percentage of non-fixed specimens are now represented in the frozen tissue biobank.

Year	2000 (before project)	866 new sample entries
	2001	938
	2002	1442
	2003	1887
	2004	2174

3. Inventory and registration of old samples.

Fresh-frozen tissue samples (focus on solid tumors) have been collected in various projects at the Department of Pathology since the 1970s. The inventory and registration of these collections of frozen tissues was completed during 2004 and the biobank database now has **23.554** entries. These samples represent an impressive range of diseased and normal tissues.

4. Database

Described in earlier reports. In the biobank database each stored frozen block or vial represents one entry. Biobank case number, patient ID number (personnummer), histopathological diagnosis, SNOMED codes (Topography and Morphology codes), patient age and sex, reference to DNA/RNA if prepared, transport time on ice, usage in research projects and freezer location is registered for each entry. A thumbnail link to the digital image of the frozen section is visible when pilot project samples are searched for. There has been no specific activity during 2004 regarding database structure.

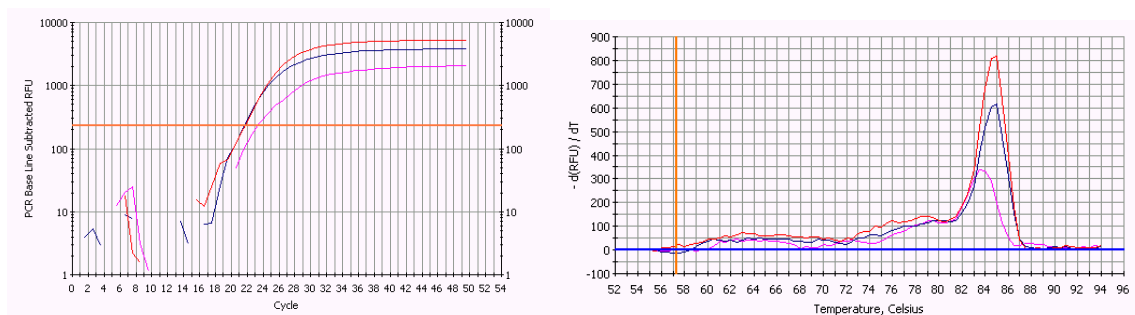
Photography of the biobank slides is lagging behind at present. In order to speed up this process we are currently testing two commercially available slide scanners (Chromavision and Scanscope) to see if high throughput automatic scanning of biobank slides is feasible.

5. Quality control – Morphology.

Described in earlier reports. New frozen section technique established (CryoJane Tape Transfer Technique). HE-stained sections have been produced from all frozen cases in the pilot biobank.

6. Quality control – DNA.

Described in earlier reports. DNA is prepared from one frozen section and the amplification efficiency of an 800 bp beta-2-microglobulin amplicon is tested by real-time PCR. The specificity of the product is evaluated by a subsequent melting point analysis.

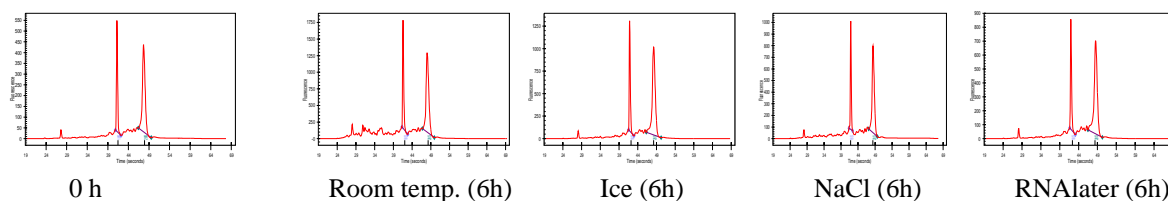


7. Quality control - RNA

RNA is prepared from 2-3 frozen sections of a sample by a phenol based method (Trizol, Invitrogen) or by a column based kit (Micro RNA Extraction kit, Qiagen). The structural

RNA-integrity (18S and 28S ribosomal RNA peaks) is then checked by microchip gel electrophoresis. We also test the expression profile of a set of genes by real-time PCR after cDNA synthesis.

During 2004 we made an effort to investigate the influence of different transport conditions and different transport times on RNA-quality in non-fixed tissue being transported between surgical departments and the pathology laboratory. These different conditions were tested in an experimental set-up using tonsil tissue.



The electropherograms showed distinct ribosomal peaks and no significant signs of degradation in non-fixed tonsil fractions transported for 6 hours at room temperature, on ice, in cold NaCl, or in RNAlater (commercial RNA-stabilising solution). A fraction of the same tonsil specimen frozen directly at the operating theatre at time point 0 h, is shown for reference (left). Expression levels of a set of genes were also tested for these conditions. Transport on ice seemed the most appropriate condition for sample transfer as expression levels changed significantly (0.5 h up to 16 h) in the other conditions tested.

We have also tested samples from the pilot biobank and RNA-quality appears to be fine in over 90 % of the samples regardless of transport time (15 min to 2 h) before freezing.

In view of these results we have relaxed the transport logistics to some extent in the pilot biobank project and we believe that it is worth while to freeze tissue from non-fixed specimens even after many hours of transport from an external hospital. A manuscript describing these data has been submitted for publication.

9. Quality assurance

During 2003 the work on a quality manual for the fresh frozen tissue biobank was initiated.

The quality manuals developed in WP 1 (Good Biobanking Practise) are acknowledged.

Additional quality control documents have been developed and implemented during 2004. JB participated in the joint work of the National Biobanking Programme and of the County Council Association, as a representative for the Uppsala-Örebro region, in the work on a

general national quality manual for biobanks (published in June 2004 -

<http://www.lf.svekom.se/artikel.asp?A=6816&C=2087>).

Biobanking Service

The pilot biobank currently serves as a biobank hotel for three external projects storing frozen tissue at our facility (inflammatory breast cancer biopsies, tendon material from chronic pain patients and peritoneal carcinosis biopsies).

We are also setting up a quality test service for external customers. Frozen tissue samples can be tested on demand with respect to morphology, DNA-, and RNA-quality (collaboration with the Mol. Path. unit.).

Delivery of samples from the fresh tissue biobank

During 2004 300 samples have been delivered to 17 different projects:

- Angiogenesis in cancer (n=10)
- Biobank quality control (n=89)
- Rejected kidney transplants (n=4)
- Genomic imprinting in cancers (n=9)
- Lymphoma in RA-patients (n=5)
- Glioma studies (n=14)
- Sarcoma genetics (n=4)
- Microdissection, cancer stroma (n=30)
- Mantle cell lymphoma (n=17)
- Lymphoma, expr. profiling (n=74)
- Tumor immunology, bladder cancer (n=2)
- Skin tumors, expr. profiling (n=15)
- Immunocytoma project (n=2)
- Pharmacogenetics, liver tissue (n=5)
- 2D-gel proteomics, cancers (n=10)
- Proximity ligation studies (n=5)
- Breast cancer, expr. profiling (n=5)

Economy

During 2004 we have kept within budget. The budget includes salary for a pathologist employed half-time (50%), salary for a full time laboratory technician, and funds for consumables.

Publications related to the pilot fresh tissue biobanking project within the national biobanking programme:

Zinc-based fixative improves preservation of genomic DNA and proteins in histoprocessing of human tissues.

Wester K, Asplund A, Bäckvall H, Micke P, Derveniece A, Hartmane I, Malmström PU, Ponten F.

Lab. Invest. (2003) 83: 889-899

Frozen Tissue Biobanking:

Stable RNA in Fresh Surgical Specimens Transported on Ice.

Botling J, Tahmasebpoor S, Ohsihima M, Östman A, Ponten F, Micke P.

Submitted.

A fluid cover medium provides superior morphology and preserves RNA integrity in tissue sections for laser microdissection and pressure catapulting.

Micke P, Bjornsen T, Scheidl S, Stromberg S, Demoulin JB, Ponten F, Ostman A, Lindahl P, Busch C.

J. Pathol. (2004) 202: 130-138.

Mutation spectra of epidermal p53 clones adjacent to basal cell carcinoma and squamous cell carcinoma.

Backvall H, Stromberg S, Gustafsson A, Asplund A, Sivertsson A, Lundeberg J, Ponten F.

Exp. Dermatol. (2004) 13: 643-650.