Report of the WP3 Expert Group on laboratory medicine

Meeting 17th and 18th December 2008

Chair:	Bill Ollier
Participants:	Bill Ollier
	Gerd Schmitz (day 1)
	Jens Hansen
	Kristian Hveem
	Martin Yuille
	Michael Neumaier
	Thomas Illig
Guests:	Gunnel Tybring (day 1)
	Erich Wichmann
Rapporteur:	Thomas Illig

Introduction

A wide range of sample types and the derivatives will be collected, processed and stored under the BBMRI umbrella. Previous biobanking initiatives and sample banks have evolved their own protocols and processes based on what was considered best practise of study design, technologies, original aims, objectives and the budgets available.

The focus of the expert group is to consider and to define a provisional set of recommendations for BBMRI on collection, processing and storage of samples. The recommendations should aim to develop best practise, harmonization and standardisation across participating centres and to minimize variation and error.

Critical issues of current technical standards

Critical issues of processing, storage, management and distribution were discussed and identified for three major sample types - DNA, Serum/Plasma and Urine/body fluids (see Table 1 and 2). Tissue banking, which is also a major part within BBMRI is not in the scope of this document/expert group.

(i) DNA extraction, storage, management (Comparison of specimens for DNA isolation and its usefulness for specific purposes are discussed in Table 1)
ii) Plasma and serum processing, management and distribution
iii) Urine other body fluids processing, storage and distribution
iv) Whole blood, viable cells, buffy coats processing, storage and distribution)

The expert group recognized that these recommendations will need further detailed elaboration and revision on a regular basis and that recommendation can only be made for some existing established technologies. The generic recommendations produced are appropriate for new population based and case-control biobanks and for existing or clinical healthcare/close to patient based biobanks. New technologies and microsampling approaches will require new specific guidelines.

Biobank infrastructure

We recommend the following basic principles, which are of special importance for the development of biobanking sample processing infrastructure.

1. Access to samples and data should be science led. Ultimately this can only be achieved through separation of downstream investigators from the process of sample/data recruitment, management and funding (UK Biobank is a good example).

This would result in improvement of sample and data quality, increased availability of downstream data for secondary analysis, a wider access to samples and data maximising research output, and in lower costs.

2. Harmonization and standardisation of processes across all components and collaborating centres can only be achieved through the implementation of a rigorous quality assurance policy which has a commitment to improvement.

Therefore regulation of Quality Assurance (QA) protocols, audit and dissemination of continuous R and D driven improvement has to be coordinated across BBMRI. Furthermore

advances in best practice should come from all BBMRI participants but have to be assessed, distributed and monitored centrally. This will avoid a drift towards a radial evolution and heterogeneity of protocols.

3. Sustainability will only be possible by developing and introducing low cost and high throughput systems. This demands web/electronic data capture, increasing automation and low energy storage.

Costs of collecting and banking of samples and data currently are too high (and getting higher) and need to be reduced, but not at the expense of quality. The major bottlenecks in studies are increasingly the costs associated with sample and phenotype collection, processing and storage rather than the downstream analysis.

Improvement of technical standards

The committee recommends the establishment of Guidelines for biobanking

- Pleural exudates
- Ascites
- Saliva
- Cerebrospinal Fluid (CSF)
- Bone marrow
- Cell lines
- Specific lymphoproliferative cells
- Synovial fluids
- Vitreous humour

Research and development in biobanking methods is required in areas including:

- Ambient temperature storage methods
- High throughput sample processing including DNA extraction
- Quality control methods for liquids including plasma, serum and urine
- EBV mediated transformation of Peripheral Blood Mononuclear Cells (PBMCs)
- Assay miniaturisation

Coordination of activities within BBMRI

The group also considered which functions within BBMRI would be best taken forward in a coordinated or centralized way. These include:

- Management
- Coordinated accrual and collection
- Audit of access processes
- Audit of consent
- Audit of experimental data return
- Prioritisation of studies
- Coordinated study design

European quality officers are needed to implement a framework comprising QA audit, protocols, protocol change and dissemination of continuous improvement, QA of experimental data accrual and Quality control of processes. Another important issue especially for new biobanks, is to develop strategies for the interoperability of tube formats, bar codes, phenotypic data, e.g. by a unique identifier system for samples and aliquots.

Table 1: Specimens for DNA isolation and their uses

Specimens	Volume	DNA yield	High density SNP-arrays	Real time PCR	Multiplex PCR Iplex method Sequenom	Genome Wide Scan CNV Methylation	Comments
Whole blood							
Whole Blood/EDTA	1-10 ml	50-500 µg (15-40 µg/ml)	YES	YES	YES	YES	
Buffy coat	1-2 ml	Mean 300µg	YES	YES	YES	YES	
Capillary blood	100-400 µl	< 2 ug	YES	YES	YES	? Depends on the yield	Low and highly variable volumes if self collection is used Tubes are not designed for storage Risk of leakage
Dried blood spots							
Whatman blood spots on filter paper		1-3 µg	NO (?)	YES	Yes, but with lower success rate and more discordance	NO	Whole-genome amplification is needed in most genotyping assays
Saliva							
Oragene DNA Self-collection Kit		Mean 30 µg Range: 0 -> 100: ≈60 % < 20 µg ≈20 % are very low	?	YES	YES	NO?	OK for SNP analysis Highly variable yield due to bacterial contamination Real Time PCR is needed for accurate human DNA quantification

Table 2: Sample types and recommended biobanking procedures

DNA	Serum / plasma	Urine
Samples should be collected, processed and stored in standard formats		
All processing and storage should be conducted in a QA certified environment using standard SOPs and QA trained staff and following good laboratory practise.		
All processing should use validated and documented methods and reagents.		
All samples received should have an associated consent record and a MTA if appropriate.		
The source material/tissue used for DNA extraction should be recorded	Any anti-coagulants and other additives should be specified.	All additives should be specified. Unless specified for a particular downstream analysis, samples should be stored without additives.
It should be specified whether the DNA is native or amplified		Spot samples should be collected mid-stream
Laboratories should aim for quality standards as specified by OECD guidelines for genetic testing		Processing should include removal of cells and particulate matter
DNA should be measured by both 260/280 spec followed by Pico green		
Samples should be collected, processed and stored in standard formats with high density bar codes		
A LIMS audit trail including all processes and events should exist for each sample and aliquot		
The electronic audit trail should start at the time and place where the sample is donated.		
A temperature record and freeze/thaw history should be associated with each sample		
Samples should be stored so as to guard against catastrophic loss.		
DNA should be stored as more than one measured stock. A policy of sample conservation/replenishment should be implemented	A policy for aliquotting should be based on conservation, minimising freeze-thaw cycles and long-term lyophilisation effects.	A policy for aliquotting should be based on conservation, minimising freeze-thaw cycles and long-term lyophilisation effects.
DNA should be stored in 10mM Tris 1mM EDTA pH 8.0 in HPLC water		
Aqueous DNA samples should be stored at -20°C or below	Samples should be stored at a temperature of -80°C or below if e.g. critical ice crystal formation is problematic	Samples should be stored at -80°C or below
Time from collection through processing should be recorded.	Time from collection through processing should be recorded. Time limits for the processing should have been defined experimentally and should be appropriate to the	Time from collection through processing should be recorded. Time limits for the processing should have been defined experimentally and should be appropriate to the

	analytes to be measured and appropriate for functional genomic analyses.	analytes to be measured and appropriate for functional genomic analyses.
Samples in research biobanks should be pseudonymised		
A method should be implemented for quality control of DNA	No recommendation can be made on sample quality control	No recommendation can be made on sample quality control
Distribution of aliquots should comply with all relevant laws and should be governed by a wide access and data sharing policy.		
Distributed material that is surplus to agreed experiments should be destroyed or, exceptionally, returned and flagged as such.		