Principles of Biobanking

6-24th June, Luxembourg
History of Biobanks

- Einstein’s brain
- Biotrade vs Biobank
- Top Models’ ovules $15K – $150K
- Petroleum $40 vs $67000 blood products
- 1951 Henrietta Lacks
- Bio-patients e.g. Amish community (U.S.)
- Bio-exhibitions
Biobank typology & evolution

Biobanking – a new scientific discipline,
Requiring the diverse technical competences
and knowledge of specialisms concerning:

- The types of materials & biospecimens preserved
- understanding the science that underpins biobank operations

‘Biospecimen science’ and ‘Science for Collections’
Biobanking Science is:

1) Inter-disciplinary (shared inter-related subjects)

2) Multi-disciplinary (non-integrative mix of subjects)

3) Pluri-disciplinary (studied across many diverse fields)

4) Tran-disciplinary (transcends conventional subject boundaries)
The Science of the Practice of Biobanking

- Evolves to meet the requirements of practitioners, patients, users, clients
- Driven by the unprecedented development of molecular, analytical and storage technologies and bioinformatics
- To meet the aspirations of their clients, biobank curators, managers and biopreservation science researchers are tasked to advance the Science of the Practice of Biobanking
- Biobanking research (Biospecimen Science) includes:
  - methods validation, to proficiency testing, to pre-analytical variables
  - understanding the fundamental basis of cryo-recalcitrance
  - critical factors affecting storage stability
  - risk management, safety and containment
And translating research outcomes into Best Practices, SOPs, and safe and secure procedures across the biobank process chain, compliant with regulatory, legal and management frameworks
Biobank Models

- **Human Biobanks** - viable & non-viable human biospecimens
- **Environmental Specimen Banks** - specimens & reference materials
- **Microbiological biobanks** - viable cultures in cryogenic storage
- **Genebanks** – conserve genetic resources in conditions that protect & preserve individuals and/or their component parts
- **Pollen and spore banks** – same principles as seed banks & vaults
- **Wildlife-veterinary genebanks** - cryostorage of animal germplasm
- **DNA banks** – clinical & non-clinical applications
- **In Vitro Genebanks** - conservation of genetic resources & germplasm in vitro
- **Cryobanks** – germplasm & viable, non-replicable resources

Seed banks and vaults - stable, environmentally controlled, hermetically sealed conditions
Scientific Collections & Conservation

- Voucher Specimens
- Type Specimens and Cultures
- Type Specimens Depository
- Living Collections
- Bioresources
“BRCs contain collections of culturable organisms, replicable parts of these, viable but not yet culturable organisms, cells and tissues as well as databases containing molecular, physiological and structural information relevant to these collections and related bioinformatics.”

BRC = Biorepository + Knowledge + Service Providers

BRCs must stringently track their bioresources and follow verified SOPs with accuracy and timeliness to ensure an effective process and supply chain.

-Purity
-Authenticity
-Stability
Cryobiology: The storage at ultra-low temperature (ca. -135°C in vapour phase down to -196°C in liquid phase) of viable and non-viable biospecimens and derivatives.

Cryoinjury: Survival after cryostorage depends on the ability of cells to overcome or avoid cryoinjury. Mazur (1965, 2004) identified two crucial factors:

• Colligative damage (excessive conc. of solutes → cell shrinkage)
• Ice (structural, osmotic damage and mechanical injury)

Cryoprotection: Protection against the damage caused by freezing. There are 2 main types of cryoprotectants:

• Penetrating or colligative (increase intracellular concentration)
• Non-penetrating / osmotic (reduce intracellular water content)
Biomaterial selection

Pregrowth Pretreatment

Cryoprotection

Cooling

Storage

Rewarming

Recovery

Viability & Competency

Stability

Cryobionomics

- Colligative
- Osmotic
- Vitrification
- Other

-135°C

-196°C

- Special media
- Light-dark regimes
- Cryoprotectant removal regime
- Other

- Vital stains
- Regrowth – regeneration – reproduction
- Development
- Motility

- Functionality
- Karyotype
- Genetic and epigenetic
Biospecimen Process Chain to End Users

1. Donor & Biospecimen
   - Area, governing authority, individual/organisation
   - Nature of donor

2. Sampling & Collection
   - Sample type, container, processing regime, ...
   - Transit, transfer, cold chain security

3. Pre-analytical & Pre-utilization
   - Sample type, container, processing regime, purification, ...
   - Culture, storage, retrieval, recovery, ...

4. Analysis & Utilization
   - Biospecimen analysis
   - Use – environmental monitoring (reference), biotechnology, breeding ...

5. Distribution
   - Sample type, container, dispatch processing regime ...
   - Transit, transfers, cold chain security
   - End point processing and performance testing regimes

- Regulatory, legal & ethical perspectives
- Risk management & mitigation
- QM, QA
- Inventories, traceability & knowledge management

End product is “fit-for-purpose”
Variability

Biological variability:
- Pre-analytical (type of sample, sampling frequency, stability)
- Analytical process
- Post-analytical process

IMP: Reference range

Pre-analytical variables:
- Patient information (e.g. gender, age, diet) [uncontrollable]
- Collection/ Phlebotomy (e.g. type of needle, Tourniquet time, blood source)
- Collection container (e.g. tube/bag, glass/plastic, gel/ non-gel separator, additives)
- Sample preparation
- Storage (e.g. duration, temperature, number of thawing events)

1. Inter-individual
2. Intra-individual
3. Pre-analytical (sample)
4. Analytical (method)
Variability

SAMPLE
- degradation
- (precision, accuracy) characteristics
- logistics

ENVIRONMENT
- decontamination
- stability of electrical power
- electromagnetic parasites
- humidity
- temperature
- vibration

MATERIALS
- reagents (conc., quantity)
- calibrators
- consumables
- cleaning
- calibrating
- manipulating
- interpreting

TECHNICIAN
- instrument qualification
- calibration
- (reproducibility, repeatability) SOP

METHOD
- variability of result
SPRECs

- SPreC = Standard Pre-analytical Code (7-element long biospecimen characterisation code)

1. Type of sample
2. Type of 1° container
3. Pre-centrifugation (delays and T)
4. Centrifugation
5. 2nd Centrifugation
6. Post-centrifugation (delay)
7. Storage (2° container & T)

E.g. fluid samples:
BLD-SED-A-B-C-D-Z
<table>
<thead>
<tr>
<th>Recommended sequence of collecting blood specimens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood culture</td>
</tr>
<tr>
<td>Non-additive glass tube</td>
</tr>
<tr>
<td>Non-additive plastic tube</td>
</tr>
<tr>
<td>With coagulation activator +/- gel</td>
</tr>
<tr>
<td>Citrate</td>
</tr>
<tr>
<td>Heparin</td>
</tr>
<tr>
<td>EDTA</td>
</tr>
<tr>
<td>Glycolytic inhibitor</td>
</tr>
</tbody>
</table>
### Blood – pre analytical variables

<table>
<thead>
<tr>
<th>Anti-coagulants</th>
<th>No anti-coagulants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Can be centrifuged immediately</td>
<td>Store for 30-45min undisturbed; centrifuge</td>
</tr>
<tr>
<td>Plasma</td>
<td>Serum</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Plasma</th>
<th>Relative centrifugal force (g)</th>
<th>Centrifugation time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelet-rich</td>
<td>150-200</td>
<td>5</td>
</tr>
<tr>
<td>Platelet-poor</td>
<td>1000-2000</td>
<td>10</td>
</tr>
<tr>
<td>Platelet-free</td>
<td>2000-3000</td>
<td>15-30</td>
</tr>
</tbody>
</table>
Sources of Errors

- Pre-analytical: 61.9%
- Post-analytical: 23.1%
- Analytical: 15%
Blood

Anti-coagulant types
www.diagnosticsample.com

Causes for alterations:
Metabolism of blood cells
Evaporation/sublimation
Chemical reactions
Microbiological decomposition
Osmotic process
Effect of light
Gas diffusion
Blood for molecular biology

Heparin at 0.05IU/reaction inhibits PCR

EDTA, ACD can inhibit restriction enzymes

EDTA, ACD, but not heparin, are removed by EtOH DNA precipitation

RBCs lysis: 155mM ammonium chloride, 10mM potassium bicarbonate, 0.1mM EDTA pH14

Nucleic acid extraction:

Prot. K releases DNA from chromatin and destroys nucleases
Saliva collection devices

Cozart oral swab (Cozart Bioscience, UK)
OraCol (Malvern Medical Developments, UK)
OraSure (Epitope, USA)
Omnisal (Saliva Diagnostic Systems, USA)
Quantisal (Immunalysis, USA)
Oragene (Genentek, USA)
Saliva sampler (Stat Sure Diagnostic Systems, USA)
Salivette (Sarstedt, Germany)
SCS saliva collection system (Greiner Bio-One, Austria)
## Storage temperatures for DNA research

<table>
<thead>
<tr>
<th>T (°C)</th>
<th>Blood</th>
<th>DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>23-25</td>
<td>1-8 days</td>
<td>DNA stable in the absence of nucleases (26 weeks)</td>
</tr>
<tr>
<td></td>
<td>Yield decreases after</td>
<td></td>
</tr>
<tr>
<td>2-8</td>
<td>3-8 days</td>
<td>DNA stable 1 year</td>
</tr>
<tr>
<td>-20</td>
<td>Not freeze whole blood before extraction; freeze cellular fractions after RBC lysis</td>
<td>DNA stable 7 years</td>
</tr>
<tr>
<td>-80</td>
<td>Min 20 years with possibility of freeze thaw cycles (min 100)</td>
<td>Min 20 years without freeze thaw cycles</td>
</tr>
</tbody>
</table>
Urine: different types of specimens

Random /spot urine: qualitative chemical determinations
First morning: cellular constituents & casts
Second morning (7-10am): quantitative determinations related to creatinine
24hr urine: quantitative determinations
Urine: influence of storage time on recovery of analytes

After 2 days:
100% decrease of citrate
30% decrease of Mg++

After 4 days:
70% decrease of Glc
40% decrease of oxalate
20% decrease of albumin
# Urine preservatives

<table>
<thead>
<tr>
<th>Preservative</th>
<th>Analytes stabilised</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thymol, 5ml of a 10% solution in propanol</td>
<td>Most constituents</td>
</tr>
<tr>
<td>Sodium azide, 10mmol/l urine</td>
<td>Glc, urea, uric acid, citrate, potassium, calcium, oxalate</td>
</tr>
<tr>
<td>HCl 25ml 6mol/l per 24 hr urine</td>
<td>Catecholamines, metabolites, calcium, magnesium, phosphate</td>
</tr>
<tr>
<td>Sodium carbonate</td>
<td>Porphyrins, urobilinogen</td>
</tr>
<tr>
<td>2g/l urine</td>
<td></td>
</tr>
<tr>
<td>Urine pH&gt;8</td>
<td>Uric acid</td>
</tr>
<tr>
<td>Urine C&amp;S tube (BD)</td>
<td>Urine particles</td>
</tr>
</tbody>
</table>
Quality

Quality = satisfaction of requirements (explicit, implicit & latent)

Comparison: Biospecimens to cars
- Aim: to win the race
- Researcher = driver → good samples
- Biobank = car dealer → need to prove good quality
Elements to check for quality of biospecimens depending on collection and end use conditions

<table>
<thead>
<tr>
<th>DNA/ RNA</th>
<th>Proteins</th>
<th>Cells</th>
<th>Cultures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purity</td>
<td>Purity</td>
<td>Quantity</td>
<td>Thawing viability</td>
</tr>
<tr>
<td>Integrity</td>
<td>Integrity</td>
<td>Viability</td>
<td>Sterility</td>
</tr>
<tr>
<td>Concentration</td>
<td>Concentration</td>
<td>Sterility</td>
<td>Authentication</td>
</tr>
<tr>
<td>Amplifiability</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cross linking</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RIN</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Some examples of biomarkers for specific pre-analytical variables:

<table>
<thead>
<tr>
<th>Application</th>
<th>Sample Type</th>
<th>QC parameter</th>
<th>Scope/ Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteomics</td>
<td>Plasma</td>
<td>Protein S</td>
<td>Storage duration</td>
</tr>
<tr>
<td>Proteins</td>
<td>Serum</td>
<td>CD40L</td>
<td>Exposure to RT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MMP-7</td>
<td>30 freeze-thaw</td>
</tr>
<tr>
<td>Posttranslational</td>
<td>RBC</td>
<td>SOD</td>
<td>5-15 freeze-thaw or 4d at 37°C</td>
</tr>
<tr>
<td>Metabolomics</td>
<td></td>
<td>MMP-9</td>
<td>Duration of storage at -80°C</td>
</tr>
<tr>
<td>Enzymes</td>
<td>CSF</td>
<td>Aβ42</td>
<td>Freeze-thaw or exposure to RT</td>
</tr>
<tr>
<td>Vitamins</td>
<td>Tissues</td>
<td>Vimentin</td>
<td>Antigenicity degradation</td>
</tr>
<tr>
<td>Oligoelements</td>
<td></td>
<td>pH</td>
<td>Tissue hypoxia</td>
</tr>
<tr>
<td>Lipids</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Ideal quality biomarker characteristics:

• Ubiquitous
• Measurable by accessible method
• On/off response
• Stable
Sample Valorisation

Use/value of samples = necessary number of samples (type of study)

Example
- Exploratory/discovery phase: less knowledge and fewer samples (e.g. 2D electrophoresis)
- Validation phase: good knowledge and many samples (e.g. candidate marker assay)

Valorising access to samples e.g. unique and guaranteed procedure

Valorisation contract: MTA
Validation

Validation - “fit-for-purpose”:

- Processing method validation
- Quality control method validation
- Biological “raw” material/derivatives validation (direct validation of samples and indirect validation of processing method)

The Key to Validation of Processing Methods:

Research in order to evaluate a number of pre-analytical variables that can potentially impact the outcome of results but are not related to inherent sample differences.

1. Identification of critical steps
2. Definition of quality attributes
3. Development of QC assays
SOPs

Definition:
Standard procedures, methods and protocols that are technically detailed and formally validated and documented. They comprise the procedures used in common by all personnel in a facility.

SOPs for what?

Requirements
- Documented
- Approved/ Authorized
- Version control (version number)
- Effective date
- Distribution list
- Ancient versions retained
- Periodically reviewed (e.g. annually)
- Electronic or paper

☑ Clear
☑ Right level of detail
**Specimen collection:**
- Collection site (/s)
- Donors

**Data collection:**
- Clinical
- Specimen
- Shipment

**Redistribution/Data Access:**
- Receive request
- Approve
- Shipment
- Provide data access

**Biorepository:**
- Sample intake
- Shipment QC
- Data collection QC
- Storage & inventory
- Shipments & Data Collection Approval

**Biorefinery:**
- Pathology QC
- Storage & Inventory
- Derivatives & aliquots
- Study-specific lab workflows

**Shipping**
Clinical research staff enters data

**Identity Matching**
Specimen/Data

**IT System**

**Public Databases**

**Quality, Traceability & Continuity**
IT – Data Management

BMS:
- Track and manage acquisitions, intake and distribution
- Collection kit management
- Shipment management
- Inventory/storage management
- Data import

Biorepository:
Sample collection, processing, distribution and storage

Sample Collection:
Clinical and sample annotation, Advanced search and Data Portal

Sample Testing:
Assay results, integrative analysis and knowledge discovery

Sample Processing:
Instrument interface and automation

LIMS:
- Track biospecimen derivatives, lab activities & annotations
- Sample processing

- Workflow management
- Instrument integration

CIMS:
- Track biospecimen derivatives, lab activities & annotations
- Sample processing
- Workflow management
- Instrument integration

- GIMS
- Data Portal & Reporting
- IAMS
- RPMS
# IBBL Policy for Cost Recovery

<table>
<thead>
<tr>
<th>Charge Band</th>
<th>Project Type</th>
<th>Direct Costs exc. Labour, e.g. courier, consumables</th>
<th>Labor inc social charges</th>
<th>Equipment Depreciation</th>
<th>Subsidised Overheads 30%</th>
<th>Full Overheads 60%</th>
<th>Comment(s) (examples)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>IBBL strategic projects</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Cancer, PD, T2DM</td>
</tr>
<tr>
<td>B</td>
<td>Feasibility studies</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Trial runs of NGS</td>
</tr>
<tr>
<td>C</td>
<td>Further projects in IBBL strategic areas</td>
<td>x</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>Collaboration Research Projects</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>Fee for service - Academic</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>Fee for service - Commercial projects</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td></td>
<td></td>
<td>x</td>
</tr>
</tbody>
</table>

**Cost Recovery**

**Profit**
Types of Contractual Relations – Clients

1) With clients – fee for service
2) With partners – research collaboration
3) With Suppliers
Transport of biological samples

Uniform Biological Materials Transfer Agreement (UBMTA)

Biosafety considerations

Dangerous Goods:
Class 6: Toxic and Infectious substances
Class 9: Miscellaneous dangerous goods
  e.g. Dry ice, GMOs

Groups 1-4 classification of micro-organisms
Shipping, Packaging

ICAO (International Civil Aviation Organisation)
IATA (International Air transport association dangerous goods)
Universal Postal Union
Rail: RID
Sea: IMDG
Road: ADR
UN 3373
Packaging

Responsibility of the sender
Docs: UN no, invoice
Packaging:
Dry ice – sufficient to stand 3 day delay
Packaging instruction
Triple package; absorbent material, label
(e.g. P650, P620)
Transporter: specialised courier company
## Classification of a substance


<table>
<thead>
<tr>
<th>Infectious Substance</th>
<th>Proper Shipping Name</th>
<th>UN number</th>
<th>Packaging Instruction</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Category A</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infectious substance</td>
<td>Infectious substance</td>
<td>2814 (human) 2900 (animal)</td>
<td>602</td>
</tr>
<tr>
<td><strong>Category B</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infectious substance</td>
<td>Biological Substance Category B</td>
<td>3373</td>
<td>650</td>
</tr>
<tr>
<td><strong>Exempt human/animal specimens</strong></td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td><strong>Patient specifications</strong></td>
<td>Infectious substance, biological substance, Category B/exempt</td>
<td>2814, 3373, none</td>
<td>602, 650, none</td>
</tr>
</tbody>
</table>
Risks & Management Tactics

**Risks:**
- Risks for biological resources (e.g. errors in processing)
- Risks for biobank staff (e.g. biological hazard & health and safety)
- Risks for donors and end users (e.g. breach of confidentiality & delays)
- Risks for the biobank (e.g. legal)

**Risk Management Tactics:**
- Duplication
- Different storage types (e.g. cryogenic and active culture)
- Stringent QA
- Formal risk assessment, mitigation and management
- Adhering to regulatory practices
Things to consider

- Appropriate QC and QA, e.g. standardised processing, recording all sample events (appropriate data management system), QC checks at every stage, periodic QC checks after storage

- Bank aliquots rather than all sample in 1 tube (reduce number of freeze-thaw cycles)

- Checks on stored samples & compare to result
Thank You for your attention