Managing Preanalytical Variables in Biobanking

Fay Betsou PhD HDR
Definition

- **3.5 biobank**
  Legal entity or part of a legal entity that performs biobanking

- **3.6 biobanking**
  Process of acquisition and storing, together with some or all of the activities related to collection, preparation, preservation, testing, analyzing and distributing defined biological material as well as related information and data
Lack of reproducibility

**SCIENTIFIC INTEGRITY**

What does research reproducibility mean?

Steven N. Goodman,* Daniele Fanelli, John P. A. Ioannidis
Sci Transl Med. 2016 Jun 1;8(341):341ps12

**ANNOUNCEMENT**

Reducing our irreproducibility

Nature 496, 398 (25 April 2013)

**RESEARCH TRANSLATION**

An incentive-based approach for improving data reproducibility

Causes of irreproducibility in the research laboratory

Nature 28 January 2016;529:456
The economics of irreproducibility in research

LP Freedman et al. PLOS Biology 2015; doi 10.1371
Before samples get on the researcher’s bench

<table>
<thead>
<tr>
<th>Source of bias</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inclusion criteria</td>
<td>Cancer subjects, male, control subjects female</td>
</tr>
<tr>
<td>Specimen collection</td>
<td>Cancer specimens from one clinic, control specimens from different clinics</td>
</tr>
<tr>
<td>Specimen processing and storage</td>
<td>Cancer specimens stored for 10 years, control specimens for 1 year</td>
</tr>
</tbody>
</table>

Adapted from J Clin Oncol 2010;28:698-704
Processing method:
Method including collection, transport, treatment and storage of biological material.
Quality Management in the processing laboratory

- **Consistency, competence**
  - ISO 20387:2018 Biotechnology-Biobanking-General requirements for biobanking

- **Critical preanalytical steps**

- **Traceability**
  - SPREC

- **Methods**
  - Manufacturer instructions
  - Validation ...
  - ISO/DIS 21899 Biotechnology-Biobanking-General requirements for the validation and verification of processing methods in biobanks.
Scope - Validation of a processing method

- Processing method, NOT analytical method
  - **NO**, Reference Materials, like the ones we use as internal QC materials in analytical methods, eg, NIST DNA
  - **YES**, spike-in Reference Materials, to be used as in-process QC materials, eg Qiagen miR39

- Processing method, NOT logistics
  - **NO**, actions with no impact on the biological material itself, eg, identification, labelling of tubes
  - **YES**, actions with impact on the biological material itself, eg, centrifugation, culture conditions
Scope - Validation of a processing method

• Validation of processing methods, NOT validation of the biological materials
  • NO, properties that have to be assessed on each unit of biological material produced, eg, representativeness / authenticity
  • YES, properties that once validated at the level of the method, one can be confident that they will meet certain specifications in all biological materials produced with the method, eg, yield, viability

• Research usage, not therapeutic
  • NO, ICH
  • YES, REMCO

It is important that any measurement performed is accurate
Validation of Processing Method

assessed by.....

• Gene expression analysis
• NGS analysis
• Metabolomic analysis
• Purity testing
• ELISPOT
• Pluripotency testing
• Growth, viability testing
• Cross contamination
• Concentration measurement
• Cell counting
• Composition testing
• Karyotyping
• Sterility testing
• Molecular integrity testing

• Fitness for purpose
• Reproducibility
• Robustness
• Homogeneity
• Stability
And more...

- In-process QC materials
- Range charts
- EQA processing schemes
Challenges

• Embedded in clinical biology laboratories?

• Exploratory methods may not get validated

• «exploratory method»
  • a method that the biobank laboratory has never performed before
  • a novel method that noone has ever performed before
  • an adaptation/modification of a known method
Other challenges

- Validation of analytical methods
- Interdependency between container-processing-analysis
- In-process QC materials
  - Homogeneity
  - Stability
  - Availability
- EQA processing schemes
  - Homogeneity
  - Stability
  - Challenging end products
  - Surrogate materials
Sample Qualification Assays


Definitions

- **Qualification**: verification of biospecimen’s fitness-for-purpose for research use - either in a specific disease area or on a specific downstream analytical platform - based on objective analytical evidence

- **Qualitative stratification**: categorisation of biospecimens into two, three or more categories, - based on objective analytical evidence - , each category corresponding to a specific *in-vivo* biological characteristic (e.g. % tumor) or to a specific *ex-vivo* pre-analytical condition (e.g. fixation time).

In the absence of scientific data on the pre-analytical robustness of a biomarker, this categorisation data can be used in future biomarker analyses as significant co-variables

- **Characterization**: clinical biology characteristics...
Quality Control of samples
## Assays for Qualification

**QC by disease**

<table>
<thead>
<tr>
<th>Biospecimen</th>
<th>Measurand</th>
<th>Disease</th>
<th>Measurement method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum, plasma</td>
<td>TNFα</td>
<td>Autoimmune, inflammatory</td>
<td>ELISA</td>
</tr>
<tr>
<td>Serum</td>
<td>Insulin C peptide</td>
<td></td>
<td>Fluoroimmuno assay, EA/RIA</td>
</tr>
<tr>
<td></td>
<td>Insulin like growth factor II precursor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma</td>
<td>Glucagon-like peptide 1 (cleared by DPP4)</td>
<td>Endocrinology and diabetes</td>
<td>EIA/RIA</td>
</tr>
<tr>
<td></td>
<td>Adenocorticotrophic hormone (ACTH)</td>
<td></td>
<td>ECLIA/RIA</td>
</tr>
<tr>
<td>Plasma, serum</td>
<td>Aldosterone</td>
<td></td>
<td>EIA</td>
</tr>
<tr>
<td></td>
<td>Somatomedin C</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
# Assays for Quality Stratification

QC for fluid biospecimens and their derivatives

<table>
<thead>
<tr>
<th>Biospecimen</th>
<th>Quality stratification parameter</th>
<th>Quality stratification parameter category</th>
<th>Measurand</th>
<th>Quality stratification threshold</th>
<th>Measurement method and reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>Post-centrifugation conditions</td>
<td>&lt;24 hrs RT</td>
<td>sCD40L</td>
<td>&gt;4ng/ml</td>
<td>ELISA</td>
</tr>
<tr>
<td></td>
<td>Coagulation conditions</td>
<td>Effectively coagulated</td>
<td>Fibrinogen</td>
<td>&lt;3mg/ml</td>
<td>ELISA</td>
</tr>
<tr>
<td></td>
<td>Hemolysis</td>
<td>Hb non-contaminated</td>
<td>Hemoglobin(Hb)</td>
<td>&lt;50mg/L</td>
<td>ELISA, spectrophotometry</td>
</tr>
</tbody>
</table>
### Assays for Quality Stratification

**QC for tissue biospecimens and their derivatives**

<table>
<thead>
<tr>
<th>Biospecimen</th>
<th>Quality stratification parameter</th>
<th>Quality stratification parameter category</th>
<th>Measurand</th>
<th>Quality stratification threshold</th>
<th>Measurement method and reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor</td>
<td>% tumor</td>
<td>Tumor-rich</td>
<td>Tumor</td>
<td>&gt;70%</td>
<td>H&amp;E staining, digital pathology</td>
</tr>
<tr>
<td>FFPE DNA</td>
<td>Fixation conditions (cross linking); extraction efficiency</td>
<td>NGS compatible</td>
<td>qPCR ΔCt</td>
<td>ΔCt &lt;2</td>
<td>Illumina FFPE QC kit</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CGH compatible</td>
<td>PCR amplicon size</td>
<td>≥200bp</td>
<td>Multiplex PCR</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Of low integrity</td>
<td>WGA score</td>
<td>≥3mg yield</td>
<td>WGA</td>
</tr>
<tr>
<td></td>
<td>DNA integrity</td>
<td>DIN</td>
<td>&gt;4</td>
<td></td>
<td>Microfluidic electrophoresis (Genomic DNA ScreenTape)</td>
</tr>
</tbody>
</table>
# Assays for Quality Stratification

**QC for cytological biospecimens and their derivatives**

<table>
<thead>
<tr>
<th>Biospecimen</th>
<th>Quality stratification parameter</th>
<th>Quality stratification parameter category</th>
<th>Measurand</th>
<th>Quality stratification threshold</th>
<th>Measurement method and reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peripheral Blood Mononuclear Cells (PBMCs)</td>
<td>Cryopreservation</td>
<td>Of high viability</td>
<td>Post-thaw viability</td>
<td>&gt;80%</td>
<td>FC; trypan blue</td>
</tr>
<tr>
<td></td>
<td>Specificity (granulocyte contamination)</td>
<td>&lt;12-14 hrs RT post-venipuncture; With no T cell function inhibition</td>
<td>CD15+ granulocytes</td>
<td>&lt;20%</td>
<td>FC</td>
</tr>
<tr>
<td>Sorted cells</td>
<td>Purity</td>
<td>Pure</td>
<td>% of cells with expected immunophenotype, eg. T cells (CD3), NK cells (CD16/56)</td>
<td>&gt;90%</td>
<td>Flow cytometry</td>
</tr>
</tbody>
</table>
Assays for Qualification

QC for fluid biospecimens and their derivatives

<table>
<thead>
<tr>
<th>Biospecimen</th>
<th>Qualification parameter</th>
<th>Measurand</th>
<th>Scope of qualification</th>
<th>Measurement method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stool DNA</td>
<td>Inhibitors</td>
<td>SPUD</td>
<td>PCR applications</td>
<td>qPCR</td>
</tr>
<tr>
<td></td>
<td>Bacterial DNA content</td>
<td>Bacterial DNA analysis</td>
<td></td>
<td>qPCR</td>
</tr>
<tr>
<td></td>
<td>Human DNA content</td>
<td>Human DNA analysis</td>
<td></td>
<td>qPCR</td>
</tr>
<tr>
<td></td>
<td>Extraction efficiency</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Initial / continual validation of processing methods
Validation of Processing Methods via Application of QC Assays

<table>
<thead>
<tr>
<th>Total NA cc’</th>
<th>ds DNA cc’</th>
<th>DNA size/integrity</th>
<th>DNA amplifiability</th>
<th>Absence of inhibitors</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOP_NA quantification_ Spectrophotometry</td>
<td>SOP_DNA quantification_ Spectrofluorimetry</td>
<td>SOP_DNA_ electrophoresis</td>
<td>SOP_long range PCR</td>
<td>SOP_SPUD assay</td>
</tr>
</tbody>
</table>

**Example**

| DNA from blood | SOP_DNA extraction from blood | >100ng/ul | >70ng/ul | MW>15kb DIN>8 | >8kb | SPUD neg |
Validation of Processing Methods via Application of QC Assays

<table>
<thead>
<tr>
<th>SOP</th>
<th>Centrifugation efficiency</th>
<th>Hemoglobin contamination</th>
<th>Blood exposure to RT</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOP_platelet counting</td>
<td></td>
<td>SOP_hemoglobin measurement</td>
<td>SOP_lacascore</td>
</tr>
<tr>
<td>SOP_plasma alipliquoting</td>
<td>&lt;10^8/ml</td>
<td>&lt;50 mg/L</td>
<td>&gt;5.2</td>
</tr>
</tbody>
</table>

2019 IBBL PT program

Schemes for Analytical Methods

✓ DNA Quantification and Purity
✓ RNA Integrity
✓ Cell Viability
✓ Tissue Histology
✓ RNA Quantification and purity
✓ Serum sCD40L
✓ DNA Integrity
The Biospecimen Fitness Guide

www.findmyassay.com
The Biospecimen Fitness Guide

www.findmyassay.com
Conclusions

- SPREC
- Initial (and continual) validation of processing methods
- Characterization, qualification, qualitative stratification of samples
  - all samples produced
  - all samples distributed
  - specific samples upon request, or “for cause”
  - randomly selected samples
Question 1

For clinical laboratories, are preanalytical processes

A. the pre-examination phase of clinical diagnostic assays?
B. the pre-examination phase of research assays?
C. processing methods, that produce specimens, and that do not necessarily belong to a “total analytical workflow”? 
Question 2

- For biobanks, embedded in clinical laboratories, and serving institutional research projects, are preanalytical processes

  A. the pre-examination phase of clinical diagnostic assays?
  B. the pre-examination phase of research assays?
  C. processing methods, that produce specimens, and that do not necessarily belong to a “total analytical workflow”? 
Question 3

For **biobanks, non-embedded in clinical laboratories**, and serving a wide range of end-users, are preanalytical processes

A. the pre-examination phase of clinical diagnostic assays?
B. the pre-examination phase of research assays?
C. processing methods, that produce specimens, and that do not necessarily belong to a “total analytical workflow”? 
Question 4

In clinical laboratories, are in-process QC materials and EQA processing schemes

A. relevant, only in the context of a specific clinical diagnostic assay?
B. relevant, independently of specific clinical or research assays?
C. relevant only for complex, challenging, exploratory, or home-made processing methods?
D. irrelevant?
In biobank laboratories,
are in-process QC materials and EQA processing schemes

A. relevant, only in the context of a specific clinical diagnostic assay?
B. relevant, independently of specific clinical or research assays?
C. relevant only for complex, challenging, exploratory, or home-made processing methods?
D. irrelevant?
THANK YOU FOR YOUR ATTENTION

Fay Betsou

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