

The use of Biological Reference Material for Biomarker Validation

Monica Marchese, PhD

Biomarker Validation Scientist

19-04-19



About IBBL

A not-for-profit public institute, dedicated to supporting biomedical research for the benefit of patients, integrated within the Luxembourg Institute of Health (LIH).

IBBL's strategy is to:

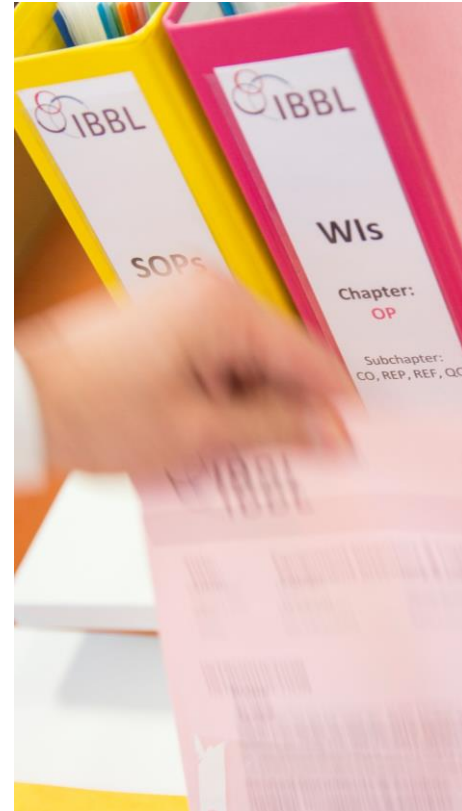
- Support Luxembourg research
- Promote biobanking and infrastructure services to medical science research internationally

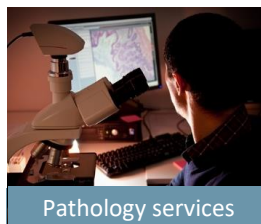
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EMPLOYEES



Dedication to Quality

- ✓ ISO 9001:2008 certification
- ✓ ISO 17025:2005 accreditation
- ✓ ISO 17043:2010 compliant



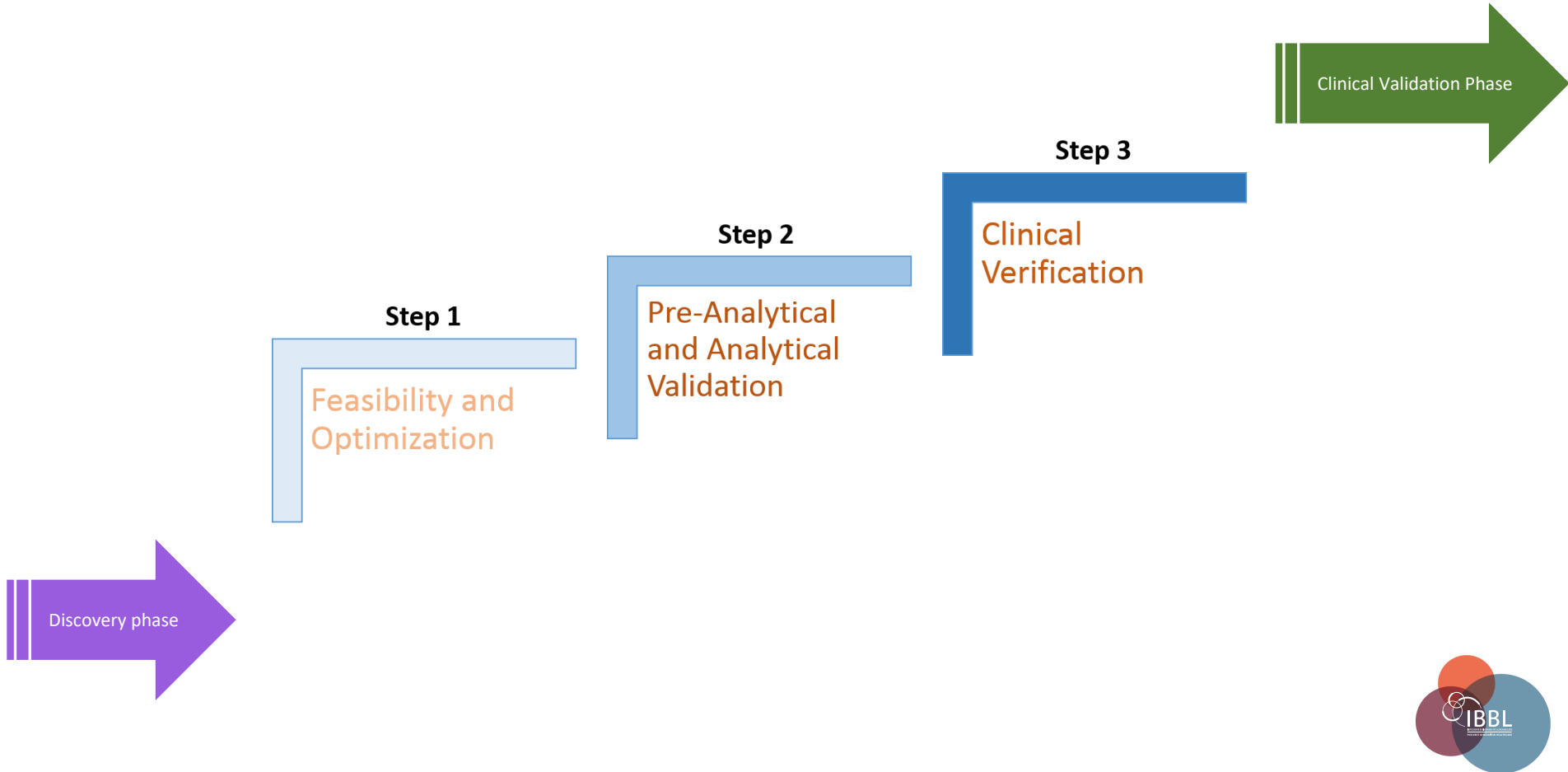


Our bioservices

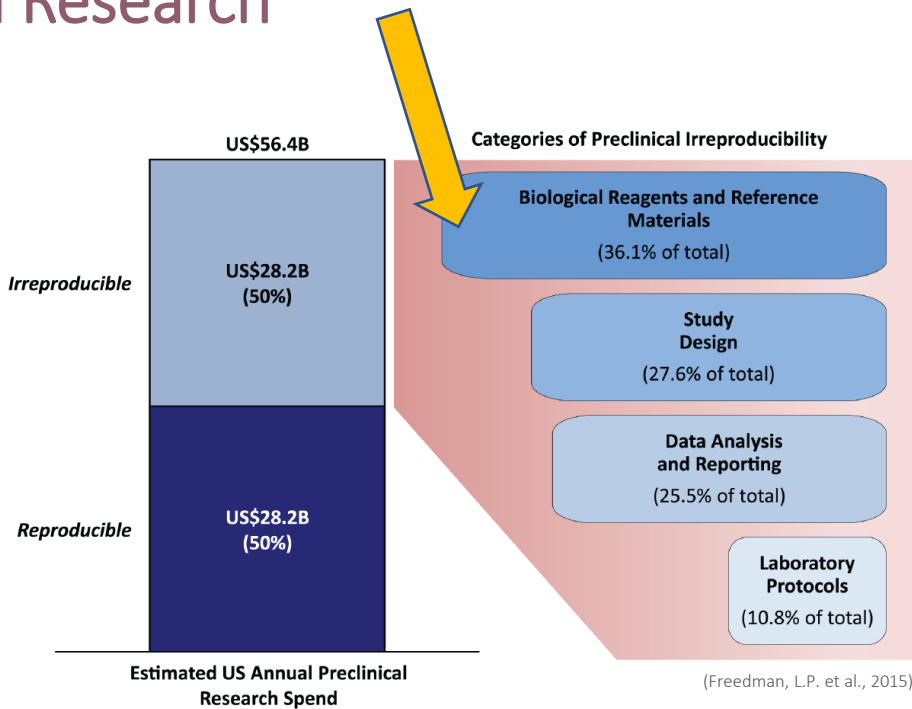
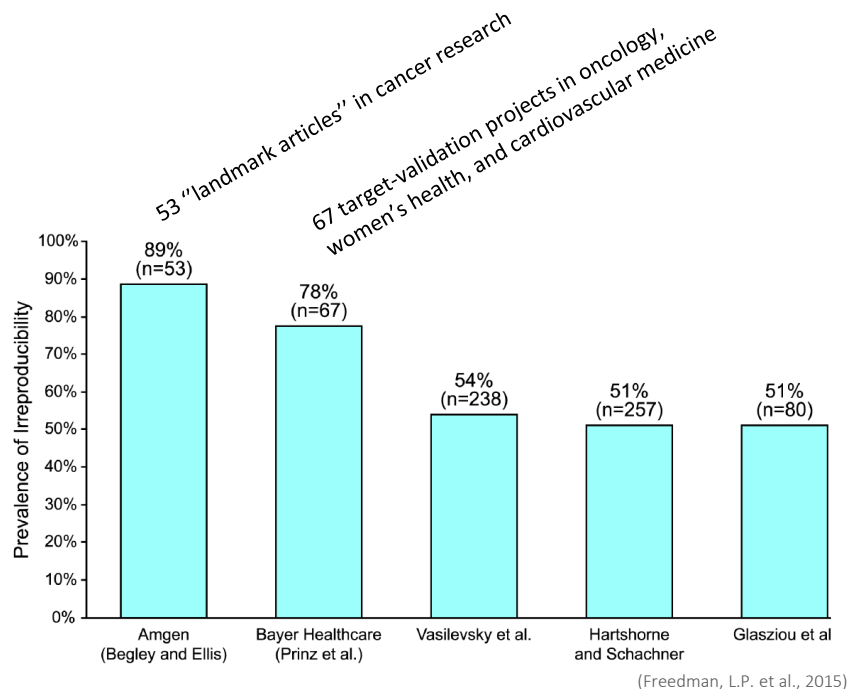
We offer a full range of biospecimen-related services to:

- Industry
- Academia
- EU consortia

RMs in the BM Value Chain



The Economics of Irreproducibility in Preclinical Research



Reference Materials (RMs)

- Two classes of RMs are recognized by ISO:
 - ✓ **Certified RMs (CRMs)**: by definition, they have to be traceable to an accurate realisation of the unit in which the property values are expressed.
Each property value *must be accompanied by an uncertainty at a stated level of confidence*
 - ✓ **Reference Materials (RMs)**: materials whose property values are sufficiently homogeneous, and well established, to be used for the calibration of an apparatus, the assessment of a measurement method, or for assigning values to materials
- Additionally, there are:
 - **Measurement RM**: for QC and validation of a quantification method
 - **Matrix RM**: adapted to the nature of the original sample to be analyzed.
They are used for QC and validation of the processing method, or the total workflow of the processing plus the quantification method

COMAR Database

Certified Reference Materials COMAR Database

Home > Reference Materials > COMAR Database

11/10/2016

Comar Database

What can we do for you?

There are many Certified Reference Materials (CRMs) operated worldwide. You will find a large share of them in our database. Try and browse our database, it is free to use. Found a suitable material? Please contact its provider directly for further info. Want to learn more about us? Please read our intro:

[→ About COMAR](#)

Quick help for your query

Searching in the COMAR database is easy, if you understand how it works. The basics are:

Start | Go to the COMAR database.

Register | We require your registration to understand whom we reach. It's quick and easy.

Search | Make any selection. Click the button [+] to confirm. If there is no [+], click the button "Apply filter".

Logical operators | You'll see A / N / O. They mean: AND, NOT, OR.

Help | Please read our helpfile. The basics are simple.

[↓ COMAR helpfile](#)

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Contact
Dipl.-Ing. Rita Pradel

Division 1.4
Branch Adlershof
Phone: +49 30 8104-5862
[Email](#)



Examples of existing RMs

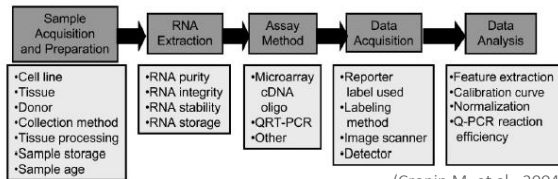


(Broothaerts W. et al., 2007)

Protein	Certified value in ERM-DA470, g/L	TF		Certified value and uncertainty in ERM-DA470k/IFCC		Components of the uncertainty budget				
		Mean of means	Relative CV, %	Value, g/L	$U_{CRM} (k = 2)$, g/L	u_{char} , %	u_{cal} , %	u_{bb} , %	u_{stv} , %	u_c , %
A2M	1.64	0.871	2.05	1.43	0.06	0.836	1.53	0.517	0.669	1.93
AAG	0.656	0.941	1.93	0.617	0.013	0.557	0.382	0.484	0.632	1.04
AAT	1.206	0.929	3.03	1.12	0.03	0.874	0.456	0.675	0.410	1.26
ALB	39.7	0.935	2.23	37.2	1.2	0.619	1.01	0.612	0.567	1.45
C3c	1.091	0.920	1.83	1.00	0.04	0.528	1.24	0.557	0.445	1.52
C4	0.151	1.070	3.02	0.162	0.007	0.873	1.66	0.418	0.497	1.98
HPT	0.893	0.995	2.26	0.889	0.021	0.627	0.504	0.637	0.494	1.14
IgA	1.96	0.918	2.36	1.80	0.05	0.632	1.02	0.340	0.357	1.30
IgG	9.68	0.948	1.37	9.17	0.18	0.368	0.516	0.425	0.615	0.98
IgM	0.797	0.908	2.11	0.723	0.027	0.636	1.44	0.745	0.562	1.83
TRF	2.45	0.964	1.60	2.36	0.08	0.483	1.22	0.498	0.646	1.55
TTR	0.243	0.906	1.80	0.220	0.018	0.543	3.71	0.880	0.592	3.89

^a The certified values are valid when the material is reconstituted according to the specified procedure. U_{CRM} , expanded uncertainty of the CRM with a coverage factor k equal to 2; u_{bb} , relative standard uncertainty related to the between-bottle heterogeneity; u_c , combined relative standard uncertainty; u_{cal} , relative standard uncertainty of the calibrant; u_{char} , relative standard uncertainty related to the characterization; u_{stv} , relative standard uncertainty related to the long-term stability of the material; u_{stv} , relative standard uncertainty related to the short-term stability of the material.

(Zegers I. et al., 2010)



(Cronin M. et al., 2004)

Table 1. Physical standards and reference materials for proteomics.

Designation	Composition	Intended use	Developer/supplier
sPRG ^{a,b}	Proteins	MS	ABRF (26)
CRM470 ^{a,c}	Proteins	Clinical laboratory	IRMM 2004 (28)
SRM 1951b ^d	Cholesterol in serum pool	Clinical laboratory	NIST (29)
RM 8327 ^e	Three peptides	Calibration	NIST

(Barker PE et al., 2006)



RMs in the lifetime of a BM: from discovery to validation

- During the discovery phase of a BM, researchers often prepare and use biological standards within their laboratory settings
- Eventually, results cannot be reproduced independently by other researchers because such standards do not reference to a widely available material
- Difficult, if not impossible, data verification between research groups, and challenging translation of the process or product into an industrial or clinical setting
- In the process of validating a BM, RCMs or RMs are needed to identify the performance of the analytical method and to demonstrate that the analytical system is operating in a state of statistical control

What RMs can Biobanks produce ?

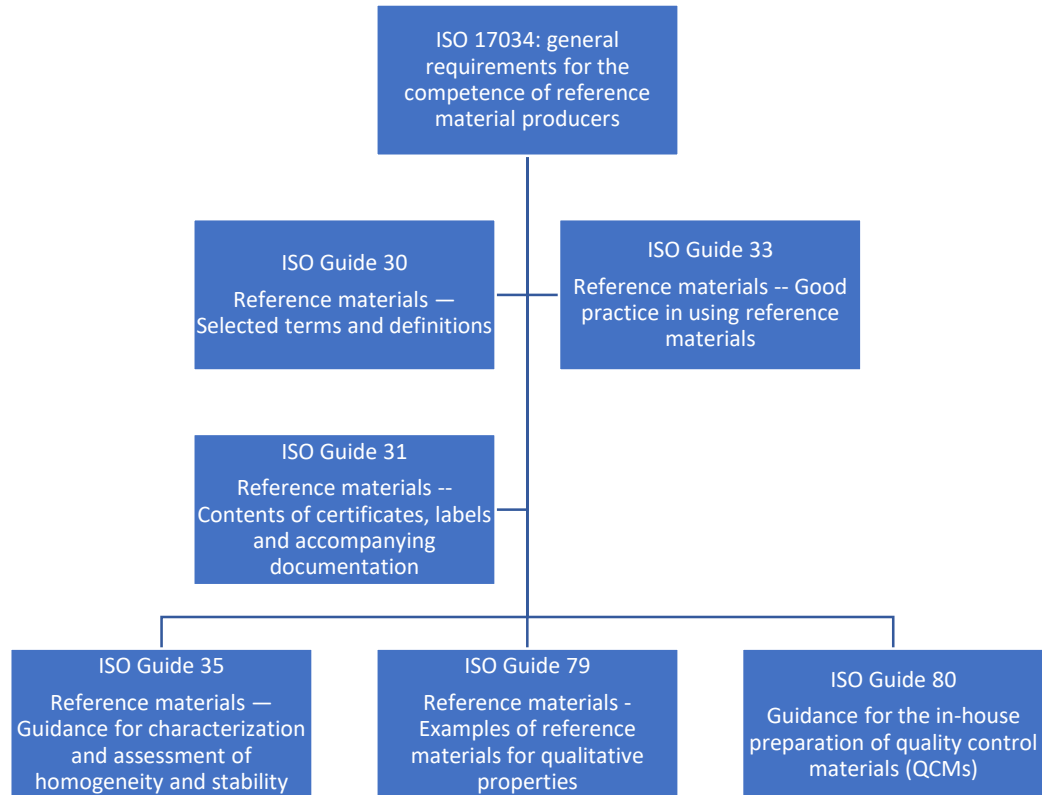
BIOPRESERVATION AND BIOBANKING
Volume 12, Number 2, 2014
© Mary Ann Liebert, Inc.
DOI: 10.1089/bio.2013.0086

Clinical Biospecimens: Reference Materials, Certified for Nominal Properties?

Fay Betsou



Standards for RM production



How do we produce RMs?

- ✓ Selection of patients
- ✓ Feasibility studies for processing
- ✓ Purity
- ✓ Characterization with respect to the targeted properties
- ✓ Fitness-for-purpose
- ✓ Homogeneity study
- ✓ Short and long term stability study
- ✓ Assignment of values and their uncertainties

Processing

↓ ↓ ↓
Standard PREanalytical Code Version 3.0

(Betsou F et al., 2018)

Surgery ▶



Specimen ▶



▶ **Fixation & Embedding**



Neutral buffered formalin



▶ **Storage**



SPREC:

TIS

Type of
samples

SRG

Type of
collection

C

Warm
ischemia
time

C

Cold
ischemia
time

NBF

Type of
fixation

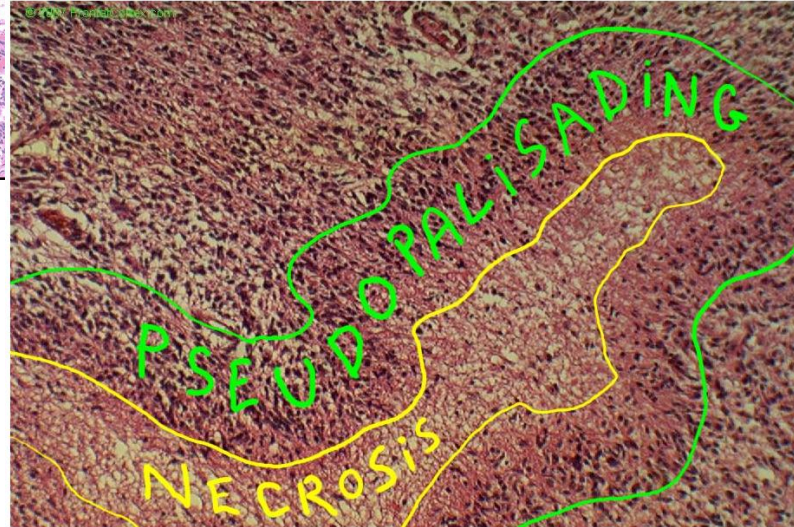
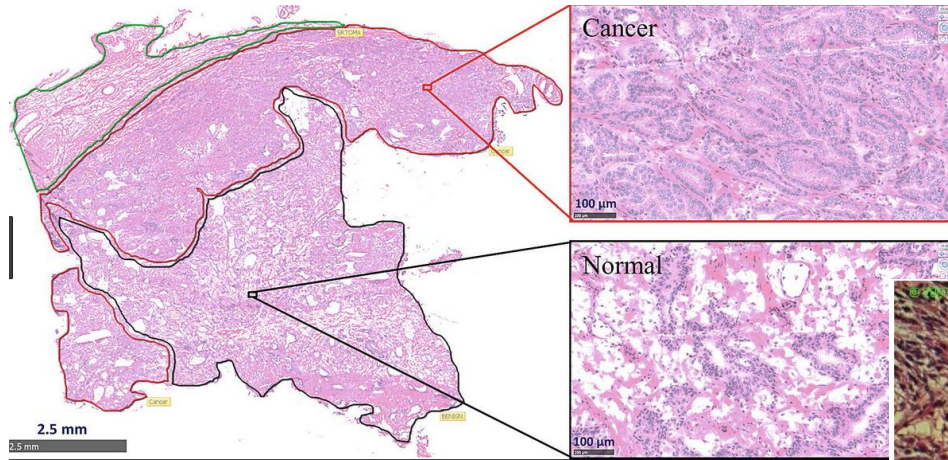
D

Fixation
time

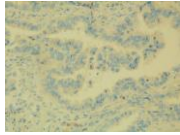
P

Container
type/temperature

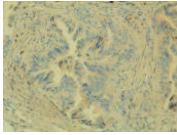
Purity



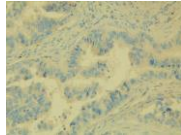
Characterization



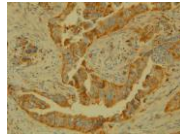
Lung ADK
CD56 20x



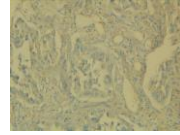
Lung ADK
Chromogranin A 20x



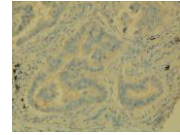
Lung ADK
CK5-6 20x



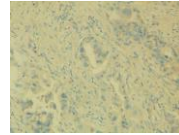
Lung ADK
Napsin A 20x



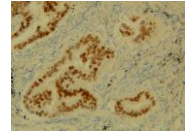
Lung ADK
p40 20x



Lung ADK
p63 20x



Lung ADK
Synaptophysin 20x



Lung ADK
TTF1 20x

Adenocarcinoma	/8140/3
Adenocarcinoma, mixed subtype	/8255/3
Acinar adenocarcinoma	/8550/3
Papillary adenocarcinoma	/8260/3
Bronchioalveolar carcinoma	/8250/3
Nonmucinous	/8252/3
Mucinous	/8253/3
Mixed <u>nonmucinous</u> and mucinous or indeterminate	/8254/3
Solid adenocarcinoma with <u>mucin</u> production	/8230/3
<u>Fetal</u> adenocarcinoma	/8333/3
Mucinous (colloid) carcinoma	/8480/3
Mucinous cystadenocarcinoma	/8470/3
Signet ring adenocarcinoma	/8490/3
Clear cell adenocarcinoma	/8310/3

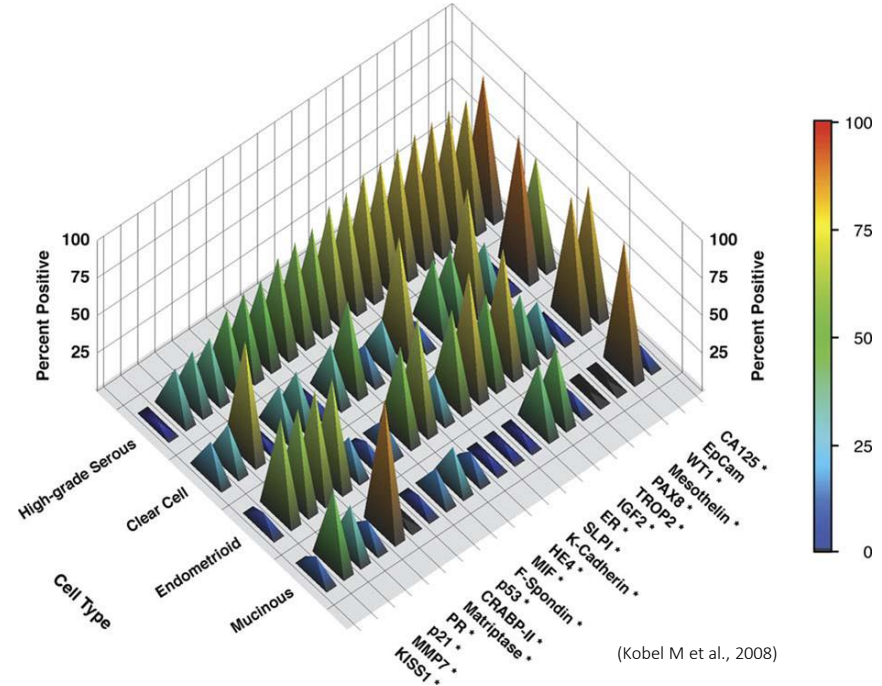
Fitness-for-purpose

Biospecimen type	Quality stratification parameter	Quality stratification parameter category	Measurand	Quality stratification threshold	Measurement method and reference
FFPE	DNA integrity / compatibility with NGS, CGH	Deamination, integrity	qPCR DCt	DCt ≥ 1.55	Illumina FFPE QC kit
		Cross linking	PCR amplicon size	$\geq 200\text{bp}$	Multiplex PCR
		Structural integrity	WGA score DIN	$\geq 3\text{mg yield}$ >3	WGA Microfluidic electrophoresis
	RNA integrity / compatibility with RNAseq	Structural integrity	DV200	$>30\%$	Microfluidic electrophoresis

ISBER BS WG, 2016

Homogeneity

- ✓ Homogeneity is the condition of being of uniform structure or composition with respect to one or more specified properties (required for RMs by ISO 17034)
- ✓ Includes both within- and between-unit homogeneity



Stability

- ✓ Stability of a biological material refers to its ability to retain its properties and performance over a specified time interval when stored under specified conditions

Value assignment

- ✓ Process of combining results from homogeneity and stability assessment with the results from the characterization studies to determine the assigned values and their uncertainties
- ✓ Total uncertainty of the assigned value
 - $\mu_{\text{CRM}} = k\sqrt{(\mu_{\text{CHAR}}^2 + \mu_{\text{BB}}^2 + \mu_{\text{LTS}}^2)}$

Microbiota samples and microbiome value assignment

b|e|i RESOURCES

SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for HM-783D

Genomic DNA from Microbial Mock Community B (Staggered, Low Concentration), v5.2L, for 16S rRNA Gene Sequencing

Catalog No. HM-783D

Product Description: A mixture of genomic DNA from 20 bacterial strains containing staggered ribosomal RNA operon counts (1,000 – 1,000,000 operons per organism per μL). **Note: The label for HM-783D is incorrect. HM-783D contains genomic DNA from microbial mock community B and not microbial mock community A.**

Lot^{1,2}: 60304010

Manufacturing Date: 31AUG2011



The challenges ^{1/2}

Rakha et al. *Breast Cancer Research* 2010, 12:207
<http://breast-cancer-research.com/content/12/4/207>



REVIEW

Breast cancer prognostic classification in the molecular era: the role of histological grade

Tubule formation – the percentage of the tumor that is made up of tubular structures

- 1 – The tumor is made up of more than 75% tubules
- 2 – The tumor is made up of 10%–75% tubules
- 3 – The tumor is made up of less than 10% tubules

Nuclear pleomorphism – the degree of change in the size and shape of the tumor cells' nuclei

- 1 – The nuclei are small and uniform in size and shape
- 2 – The nuclei are medium to large in size, but are mostly the same size and shape
- 3 – The nuclei are large and vary in size and shape

Mitotic count – the number of cells that are actively dividing

- 1 – The tumor cells are dividing at a slow rate
- 2 – The tumor cells are dividing at a moderate rate
- 3 – The tumor cells are dividing at a fast rate

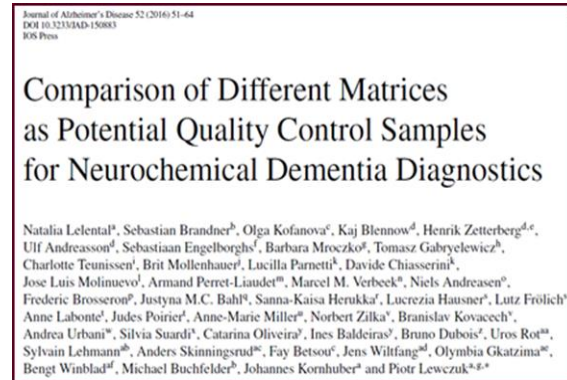
Bloom-Richardson Grade – Nottingham modification

Grade	Combined Score	Description
I	3–5	Low-grade (well-differentiated) tumours that do not appear to be growing quickly and are less likely to spread
II	6–7	Intermediate-grade (moderately differentiated) tumours that have features between grade 1 and 3
III	8–9	High-grade (poorly differentiated) tumours that tend to grow faster and are more likely to spread



The challenges ^{2/2}

- ✓ Define acceptance criteria for homogeneity, stability, total uncertainty
- ✓ Establish traceability to SI
- ✓ Standardized pathology and clinical data
- ✓ Validate the analytical methods used
- ✓ High level of homogeneity and stability of both the matrix and the analytes
- ✓ Volumes ; surrogate materials





THANK YOU FOR YOUR ATTENTION

Monica MARCHESE

PhD

Monica.Marchese@ibbl.lu



www.ibbl.lu

