Improve human-derived sample quality grade in European Biobanks: focus on snap frozen tissues

(BBMRI-ERIC WG 1 in the Work plan *Quality*)

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Content

- Goals of the WG-1
- Technical Framework
- Overview of the participation in the WG-1
- Pre-An Evaluation Tool
- Next steps

Pre-analytical conditions matter !

The NEW ENGLAND JOURNAL of MEDICINE

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70-Gene Signature as an Aid to Treatment in Early-Stage Breast Cancer

F. Cardoso, L.J. van't Veer, J. Bogaerts, L. Slaets, G. Viale, S. Delaloge, J.-Y. Pierga, M. DeLorenzi, A.M. Glas, V. Golfinopoulos, T. Goulioti, S. Knox, E. Matos, B. Meulemans, R. Passalacqua, P. Ravdin, I.T. Rubio, M. Saghatchian, T.J. Smilde, C. Sotiriou, L. Stork, A.M. Thompson, J.M. van der Hoeven, P. Vuylsteke, R. Bernards, K. Tryfonidis, E. R. for the MINDACT Investigators*

Breast cancer molecular profiling with single s predictors: a retrospective analysis

Britta Weigelt,* Alan Mackay,* Roger A'hern, Rachael Natrajan, David S P Tan, Mitch Dowsett, Alan Ashworth,

Summary

Background Microarray expression profiling classifies breast cancer into five molecular sub basal-like, HER2, and normal breast-like. Three microarray-based single sample predict define molecular classification of individual samples. We aimed to establish agreeme identification of breast cancer molecular subtypes.

Methods Previously described microarray-based SSPs were applied to one in-house (n=53 (n=779) breast cancer datasets. Agreement was analysed between SSPs for the whole cla five molecular subtypes individually in each cohort.

Findings Fair-to-substantial agreement between every pair of SSPs in each cohort was re the five molecular subtypes, only basal-like cancers consistently showed almost-perfec proportion of cases classified as basal-like in each cohort was consistent irrespective of proportion of each remaining molecular subtype varied substantially. Assignment of in luminal B, HER2, and normal breast-like subtypes was dependent on the SSP used. The with outcome of each molecular subtype, other than basal-like and luminal A, varie However, different SSPs produced broadly similar survival curves.

Interpretation Although every SSP identifies molecular subtypes with similar survival, th same patients to the same molecular subtypes. For molecular subtype classification to clinical practice and treatment decision making, stringent standardisation of metho identification of breast cancer molecular subtypes is needed.

Although microarray-based gene expression profiling analysis has been reported as able to provide reasonably reproducible results for molecular classification of breast cancer,[3] and [4] our findings show that without thorough standardisation, these tumours cannot be classified reliably by this approach. As emphasised by Ioannidis and colleagues,[35] other investigators might only be able to predict molecular subtypes accurately when a detailed description of data processing and analytical methods is provided. A roadmap similar to the one described for development and validation of therapeutically relevant genomic classifiers[36] is needed for introduction of breast cancer molecular taxonomy in clinical practice. Furthermore, careful standardisation of preanalytical variables that have a direct effect on expression profiles, such as stromal component[28] and tissue processing,[37] are equally crucial for development of reliable and reproducible classifiers.

Content

- **Goals of the WG-1**
  - ✓ to develop evidence-based quality criteria
  - ✓ to implement new European quality standards
- Technical Framework
- Overview of the participation in the WG-1
- Pre-An Evaluation Tool
- Next steps

Content

- Goals of the WG-1
- Technical Framework
  - ✓ self assessment tool built into BBMRI-ERIC website
  - ✓ use of CEN/TS[#] for comparing SOPs used in the different BBMRI-ERIC biobanks & of the IT tool developed by BBMTI.at
- Overview of the participation in the WG-1
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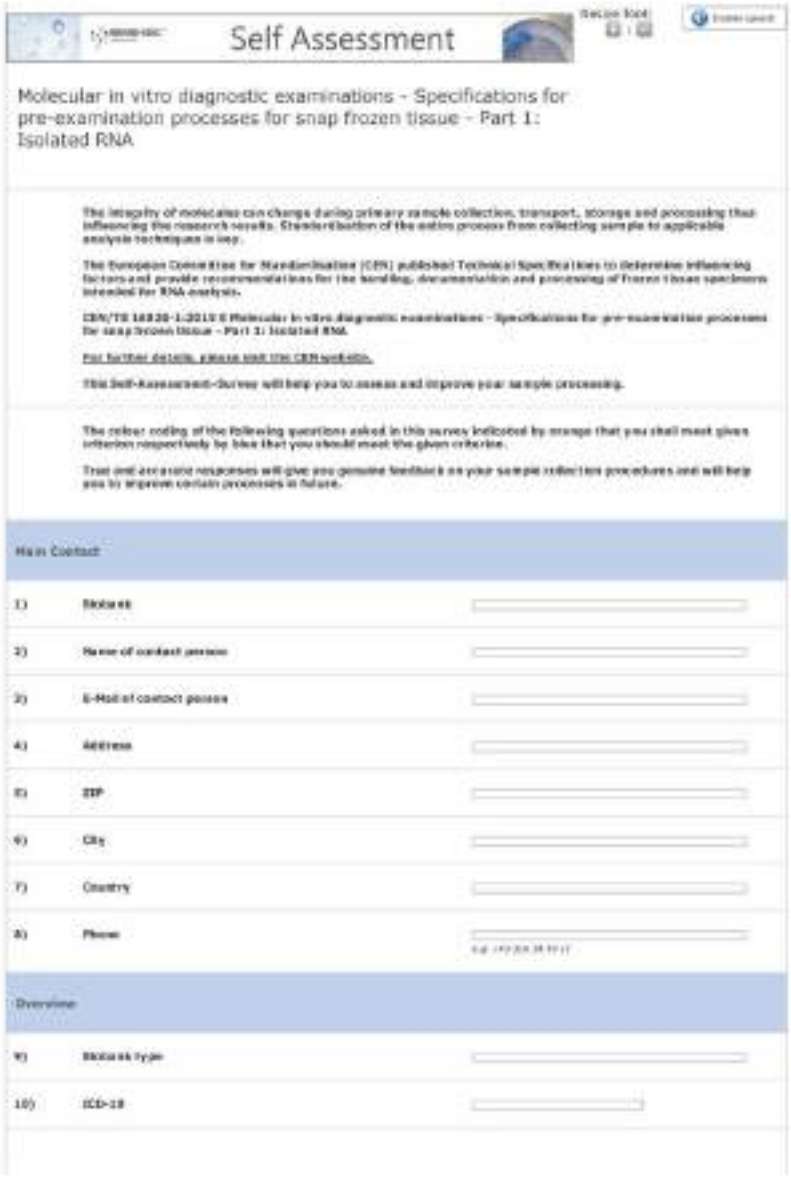
- Next steps

[#]Technical Specification (TS) of the European Committee for Standardization (CEN)

Self assessment survey



Self Assessment

Molecular in vitro diagnostic examinations - Specifications for pre-examination processes for snap frozen tissue - Part 1: Isolated RNA

The integrity of molecules can change during primary sample collection, transport, storage and processing thus influencing the research results. Standardisation of the entire process from collecting sample to applicable analysis techniques is key.

The European Committee for Standardisation (CEN) published Technical Specifications to determine influencing factors and provide recommendations for the handling, documentation and processing of Frozen tissue specimens intended for RNA analysis.

CEN/TS 16820-1:2015 Molecular in vitro diagnostic examinations - Specifications for pre-examination processes for snap frozen tissue - Part 1: Isolated RNA.

For further details, please visit the CEN website.

This Self-Assessment-Survey will help you to assess and improve your sample processing.

The colour coding of the following questions asked in this survey indicated by orange that you shall meet given criterion respectively by blue that you should meet the given criteria.

Your and anonym responses will give you genuine feedback on your sample collection procedures and will help you to improve certain processes in future.

Main Contact

1) Biobank

2) Name of contact person

3) E-Mail of contact person

4) Address

5) ZIP

6) City

7) Country

8) Phone

Overview

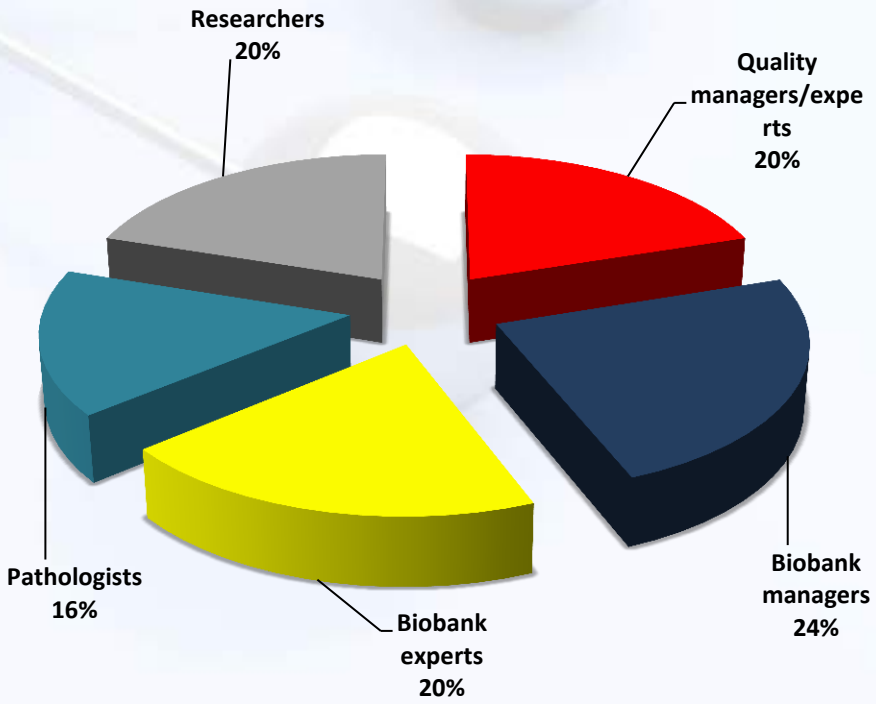
9) Biobank type

10) ICD-10

Participation of BBMRI-ERIC Experts in the WG-1 for snap frozen tissues

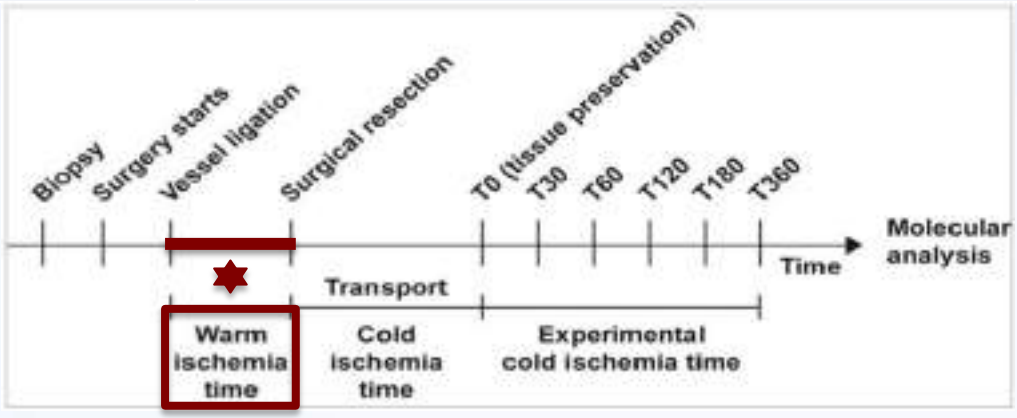
- 26 Participants from 13 BBMRI national nodes
  +
- Participants from BBMRI-ERIC HQ

- WG meetings (TCs, Webinars, 3 web conferences sharing comments on *Research Electronic Data capture* Tool and on *CEN/Technical Specification* survey)



Check-list items based on snap frozen tissue RNA-Part 1 (CEN/TS 16826-1)

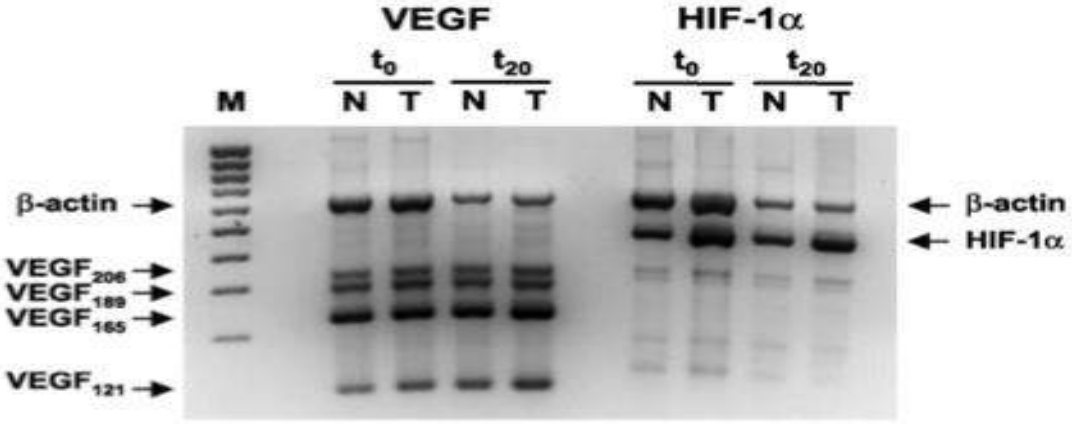
Outside the laboratory	Content
Primary sample donor/patient *(shall)*	Donor/patient ID, health status, routine and/or special medical treatment
Primary tissue sample *(should)*	Time of removal from the body, tissue type description, cryo-storage conditions, **warm ischemia**
Primary tissue processing (shall)	Additions/modifications, transport container and relative labeling, stabilization procedures
Transport requirement (shall)	Protocol for transport procedure, **cold ischemia**



Courtesy of K.F. Becker

Instantaneous response to surgically induced hypoxia of hepatocellular carcinoma

Letters to the Editor / Journal of Hepatology 40 (2004) 559–565



	Time (min)	HIF-1α mRNA (NDU)	$VEGF_{165}$ mRNA (NDU)
Parenchyma	t_0	0.67	1.44
	t_{20}	1.87	4.00
HCC	t_0	1.17	1.26
	t_{20}	3.16	6.03

Tumor tissue and non-cancerous perenchyma are both very sensitive to warm ischemia induced during surgical procedures.

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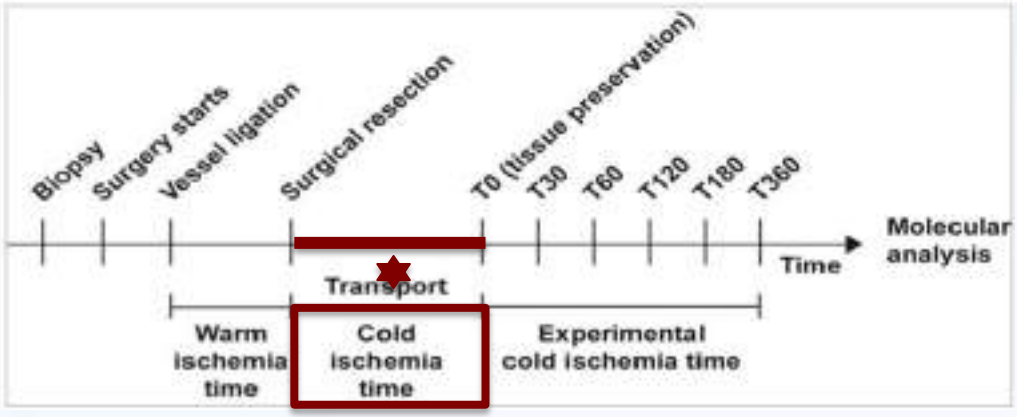
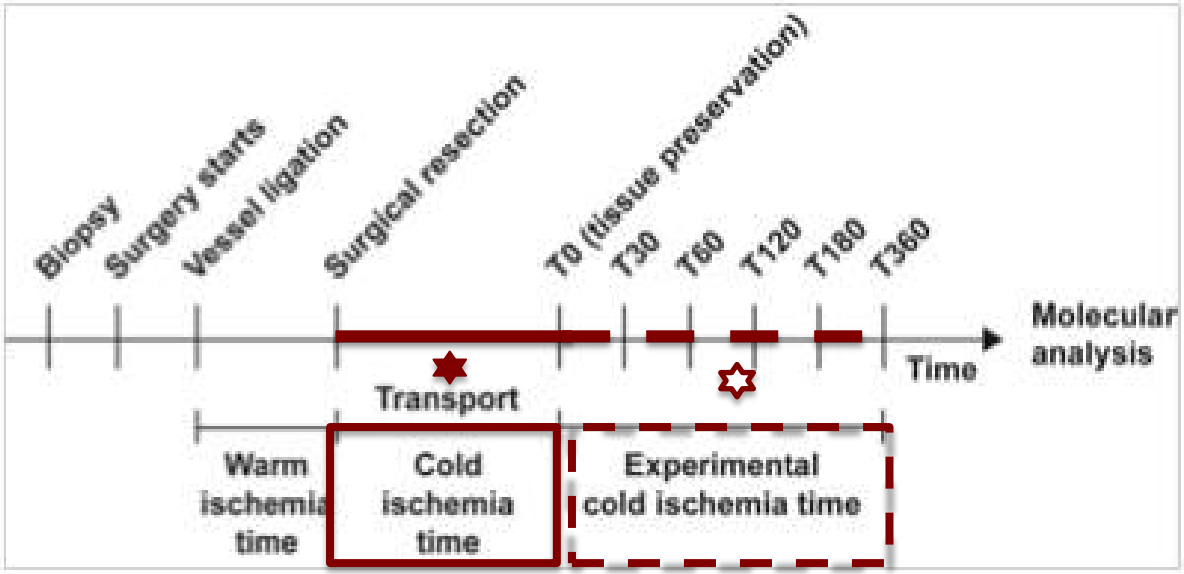


Courtesy of K.F. Becker

Unique tissue collection in the TUM biobank

(experimentally delayed tissue preservation)



Analysis of:

- **Proteins**
- **Phosphoproteins**
- **mRNA**
-

1411 samples from 128 patients
(935 frozen, 476 FFPE)
(26 September 2015)

Cryo	Tumour	495
	Normal / Reference	440
FFPE	Tumour	252
	Normal / Reference	224

Tumor tissues:

- 30 colon cancers
- 15 gastric cancers
- 16 liver cancers
- 18 pancreas cancers
- 8 rectum cancers
- 9 oesophagus cancers
- + 32 others

munich biotech

SPIDIA

Courtesy of K.F. Becker

Technische Universität München

How to control cold Ischemia: *Applicability of Under Vacuum Fresh Tissue Sealing and Cooling to Omics Analysis of Tumor Tissues (Biopreservation & Biobanking, August 2016)*

DOI 10.1007/s10561-007-0529-x

LETTER TO THE EDITOR

Tissue transfer to pathology labs: under vacuum is the safe alternative to formalin

Gianni Bussolati · Luigi Chiusa · Antonio Cimino · Giuseppe D'Armento

Eliminate la formalina dalle sale operatorie!

TissueSAFE

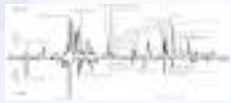
Sistema di trasferimento ad ultravuoto per campioni biologici

Validation of under vacuum sealing (UVS) on:

Histo-morphology

Gene expression profiling (GEP)

Metabolic content



Peptidome analysis

a panel of human normal and cancer specimens *(21 cases including breast, colon, lung cancer and mesenchymal tumors)*, rapidly dissected after surgery, divided in tissue aliquots, UV sealed, maintained at 4° C for 1-24-48-72 hours and compared to the tissue snap-frozen at t=0

Morphology (*H&E*) **and immunohistochemical reactivity** *(Vimentin, Ki67)* were perfectly preserved and unaltered over the entire observation period

Fresh tissues may be conserved for longer periods thus permitting transport of fresh surgical specimens from distant institutes

GEP (*Illumina Human HT12_v4 Platform)*: Gene ontology statistical analysis reveals that some pathways or processes could be deregulated over time even if the analysis of single genes did not show significant results

Proteomic studies showed that:

- ✓ protein degradation does not significantly affect SELDI-TOF MS profiles at different storage times
- ✓ quantity and phosphorylation measures of mTOR protein are generally related to cellularity
- ✓ tissue heterogeneity emerged as an important source of variability in protein expression results

Overall 24 h may be considered an optimal limit to provide a comprehensive proteomic information in protein extracts

Metabolomic studies (*High-resolution NMR)* showed that changes occurred :

- ✓ after 1h of UV storage in breast and lung cancer tissues under UVS
- ✓ after 24h in normal and colon cancer tissues under UVS

Veneroni S. et al., Biopreservation and Biobanking, 2016

Check-list items based on snap frozen tissue RNA-Part 1 (CEN/TS 16826-1)

Inside the laboratory	Content
Primary tissue receipt *(shall)*	Name of person, arrival time, conditions
Pathological evaluation of the specimen *(shall)*	Board certified Pathologist (*or other qualified personnel)*, different options for sample selection, check of patient/donor information
Cryo-storage of the specimen (shall)	Freezing procedures, time point and date, size and selection of sample container for cryo-storage and relative labeling
Storage requirement (shall)	Temperature monitoring and alarm systems, back-up procedures, retrieval times, accident documentation
Isolation of total RNA (shall)	*Morphology check by H&E slides*, use of commercial kits, use of validated laboratory own procedures, documentation of protocol violations
Quality assessment of isolated RNA (shall)	Quality, quantity and integrity check (A_{260} absorption or spectrofluorometry, A_{260}/A_{280} ratio, RNA integrity)
Storage of isolated RNA (shall)	Kit provider's specification, verified procedures, appropriate storage temperature and cryovials

Content

- Overview of the participation in the WG-1
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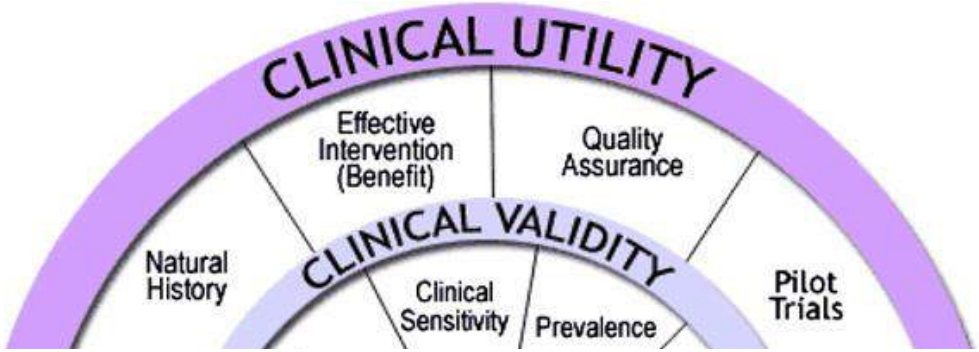
- **Pre-An Evaluation Tool & Next steps**

TEMPLATE					A				
Based on: snap frozen tissue RNA-Part 1 (CEN/TS 16826-1)		BASIC y/p/n	ADVANCED with Specific Sample Data		BASIC y/p/n		ADVANCED with Specific Sample Data		
		y = yes, p = partially, n = no	If yes (in column J) -> enter explicit data in columns L,M,N); if NO -> enter info: why not						
Outside the laboratory		y/p/n			y/p/n		Sample data		
Collection of tissue		n/p/n	CRYO	Source of data	Y		CRYO	FFPE	FFPE
Information about sample donor			CRYO	Source of data			CRYO	FFPE	FFPE
Donor/Patient ID documented	e.g. code	n/p/n	<enter ID code>		Y	points	Patient ID number (hospital code)		
Health status of donor/patient documented	e.g. healthy, disease type, concomitant disease	n/p/n	<enter text>		P	points	Only Pathology data		
Medical treatment documented	e.g. anaesthetics, medications, surgical or diagnostic procedures (e.g. biopsy device used for the collection);	n/p/n	<enter text>		n	points	can be requested but first FT ischemia time		
Start of warm ischemia documented	Surgery: Date of vessel ligation/arterial clamping time	n/p/n	<enter dd.mm.yyyy>		p	points	FT in request		
	Surgery: Time of vessel ligation/arterial clamping time	n/p/n	<enter hh:mm>		p	points	FT in request		
Information on the primary tissue sample			CRYO	Source of data			CRYO	FFPE	FFPE
Cold ischemia (start) documented	Date of tissue removal from body	n/p/n	<enter dd.mm.yyyy>		y	points	Y	Y	<enter
	Time of tissue removal from body	n/p/n	<enter hh:mm>		N	points	Requested	N	<enter
Tissue type and condition documented	General tissue type and condition	n/p/n	<enter text>		y	points	<enter text>		
	Organ of origin + location within	n/p/n	<enter text>		y	points	<enter text>	<enter text>	<enter text>
Type and start of fixation (if started outside the biobank) documented	Date of start	n/p/n	<enter dd.mm.yyyy>		y	points	<enter text>		
	Time of start	n/p/n	<enter hh:mm>		n	points		<enter	<enter
	Fixative type	n/p/n	<enter text>		n	points		<enter hh:mm>	<enter hh:mm>
	Fixation condition	n/p/n	<enter text>		y	points		<enter text>	<enter text>
Information on the primary tissue sample processing			CRYO	Source of data	n	points		<enter text>	<enter text>
Modifications after removal from body documented	e.g. labelling for specimen orientation such as ink-marking, stitches, incisions	n/p/n	<enter text>				CRYO	FFPE	FFPE
Selection/use of transport containers performed	e.g., cooling box, vacuum packaging, ...	n/p/n	<enter text>		y/p/n	points	<enter text>		
Selection/use of stabilisation procedures for transport of unfixed primary tissue performed	e.g., cooling methods, fixation	n/p/n	<enter text>		y/p/n	points	<enter text>		
Labelling of the transport container performed	e.g., registration-no., barcode (2D or 2D), primary sample type, quantity, and organ tissue of origin);	n/p/n	<enter text>		y/p/n	points	<enter text>		
	e.g. documented when several aliquots of a single sample with different fixtures are in one container	n/p/n	<enter text>		y/p/n	points	<enter text>		
Transport requirements		n/p/n	CRYO	Source of data					
Transport procedure protocol established		n/p/n	<enter link>		y/p/n		CRYO	FFPE	FFPE
Compliance with transport protocol / deviations documented	Transport of unfixed primary tissue performed on wet ice or 2-8 °C without delay	n/p/n	<enter text, if measu rest temp. °C>		y	points	y		

Key points

- Biomarkers are essential tools for personalized medicine (and health economics), and are crucial to improve the success rate of new therapies
- Implementation of biomarkers into clinical practice presents biological, clinical, technical and logistic challenges, with special reference to standardization across multiple Countries and clinical practices
- Robust lab methodologies are mandatory at all analytical phases of biomarker development, from pre-analytical (sample definition, handling and processing) to analytical (data and QC recording) and post-analytical (data interpretation and reporting)
- The activity of the WG-1 of BBMRI-ERIC will provide functional/operative recommendations to increase biomarker reliability and foster the development of a Precision Medicine ultimately useful to provide benefit to patients



The best preparation for tomorrow is to do today's work superbly well

Sir William Osler
Canadian Physician (1849-1919)