UNIVERSITÀ
DEGLI STUDI DI TRIESTE

BIOMARCATORI NEI TESSUTI

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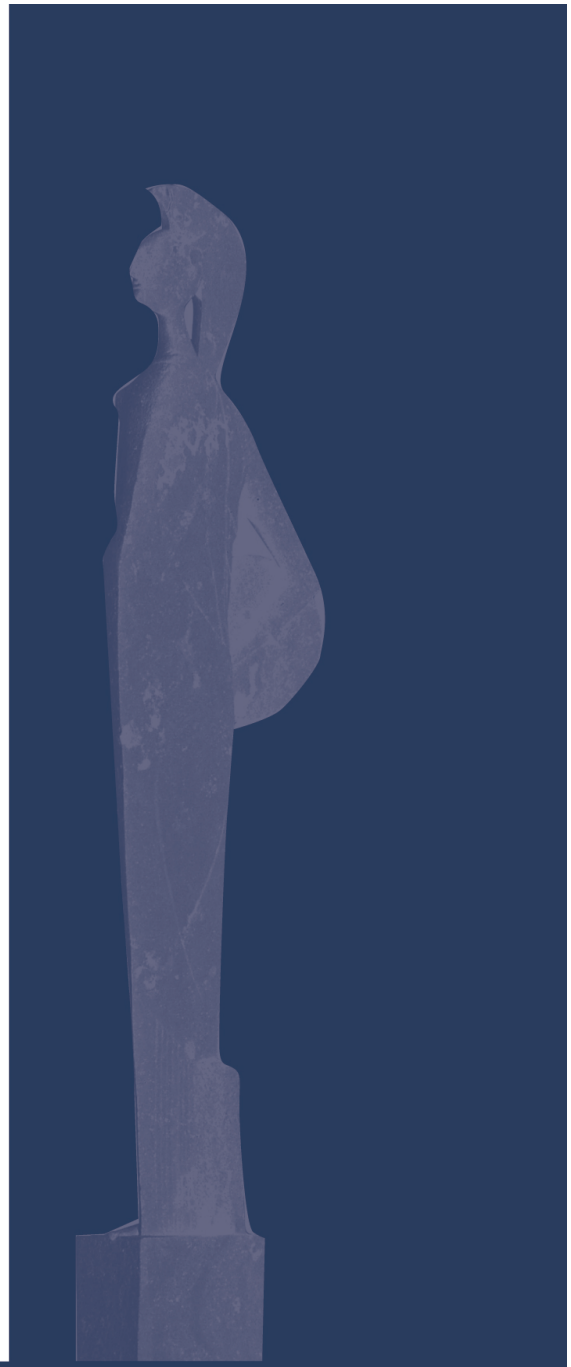
UNIVERSITÀ
DEGLI STUDI DI TRIESTE

- Quality of Clinical samples
- Clinical tissues
- Biomarkers' definition and classification
- Analytical Methods



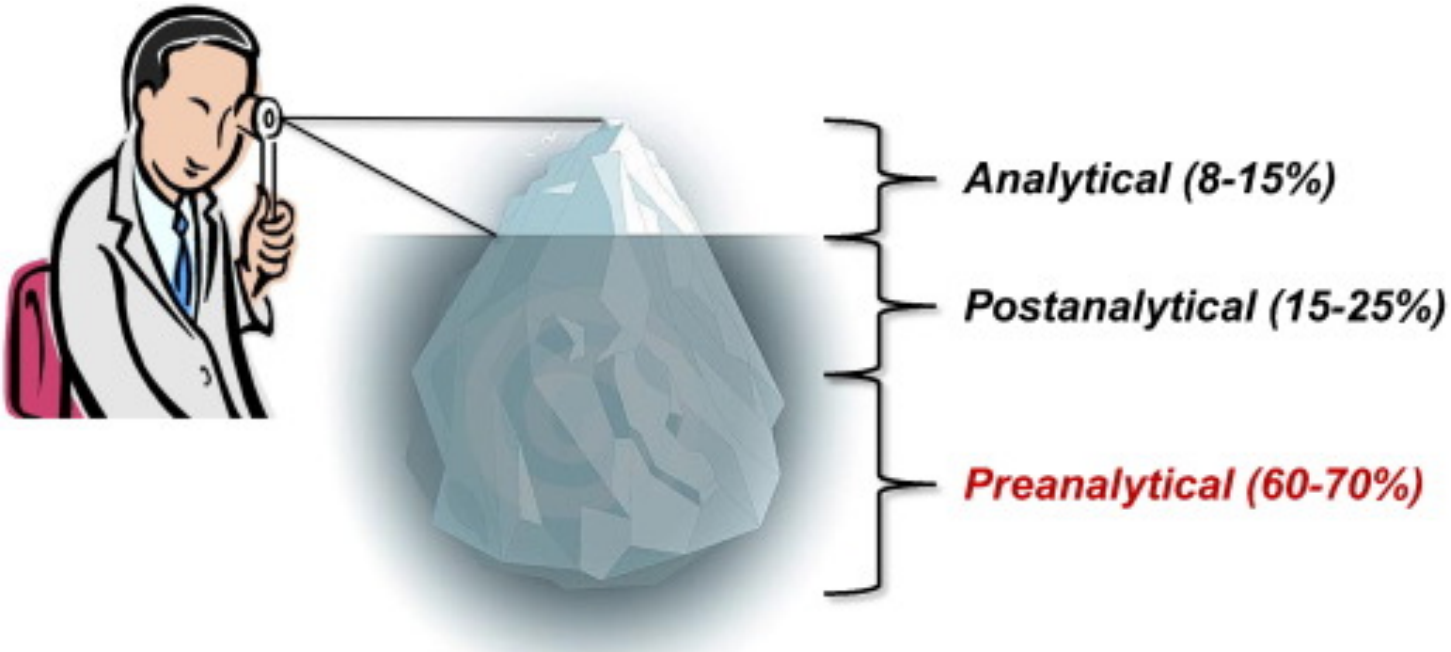
Sources of Clinical research and Diagnostic Variabiliy

- ✓ Tissue and macromolecule pre-analytical preservation
- ✓ Heterogeneity at the clinical, morphological or molecular level
- ✓ Selection and standardization of analytical procedures
- ✓ SOPs

What are pre-analytical conditions?
How do they affect analytical results?
How can quality of samples be assured?

Why pre-analytics?

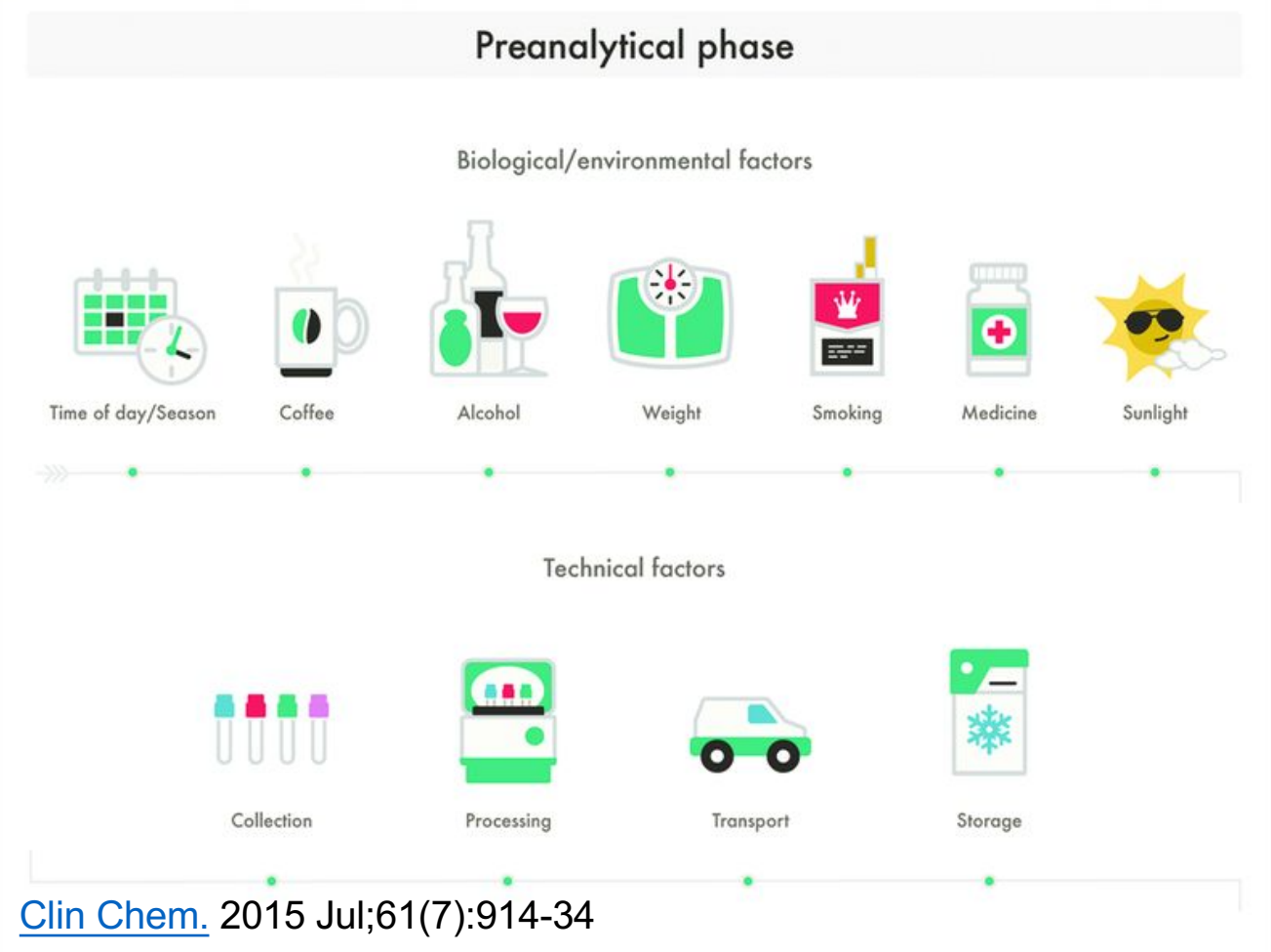
- Physicians rely on accurate laboratory test results for diagnosis and guiding therapy: more than 70% of clinical decisions are based from information derived from laboratory results (MLO Med Lab Obs. 2014 May;46(5):22, 24, 26)
- 10^7 € of funding may be lost each year in clinical trials in the EU due to pre-analytical and analytical problems (Ann Transl Med. 2016 May;4(9):181)



Clin Biochem. 2016 Dec;49(18):1313-1314

What is pre-analytics?

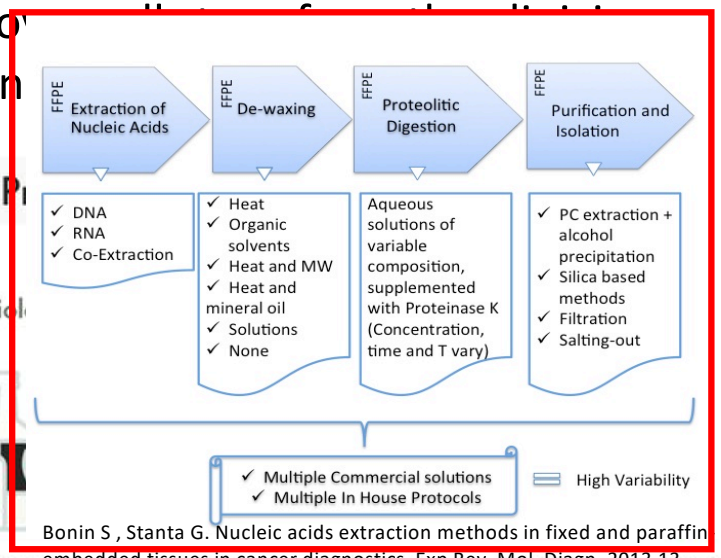
Pre-analytical phase: covers all steps from the clinicians requests to the beginning of the analytical examination, included nucleic acid or protein extractions



Clin Chem. 2015 Jul;61(7):914-34

Why extractions into pre-analytics?

Pre-analytical phase: co… …uests to the beginning of the analytical examin… …in extractions



Bonin S , Stanta G. Nucleic acids extraction methods in fixed and paraffin embedded tissues in cancer diagnostics. Exp Rev Mol. Diagn. 2013,13.

Clin Chem. 2015 Ju…

Serena Bonin, Falk Hlubek, Jean Benhattar, Carsten Denkert, Manfred Dietel, Pedro L. Fernandez, Gerald Höfler, Hannelore Kothmaier, Bozo Kruslin, Chiara Maria Mazzanti, Aurel Perren, Helmuth Popper, Aldo Scarpa, Paula Soares, Giorgio Stanta and Patricia JTA Groenen."MULTICENTRE VALIDATION STUDY OF NUCLEIC ACIDS EXTRACTION FROM FFPE TISSUES" Virchow Arch 2009

Why pre-analytics?

- Standardization of pre-analytical processes is key to guarantee reliability of analytical results
- Same requirements for diagnostics and biobanks
- Increasing demand in the context of personalized medicine and companion diagnostics

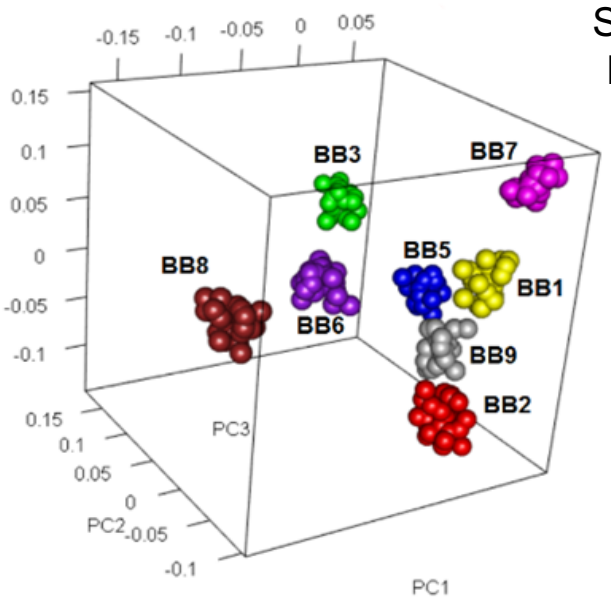
European healthy subjects

EDTA-plasma from 9 biobanks

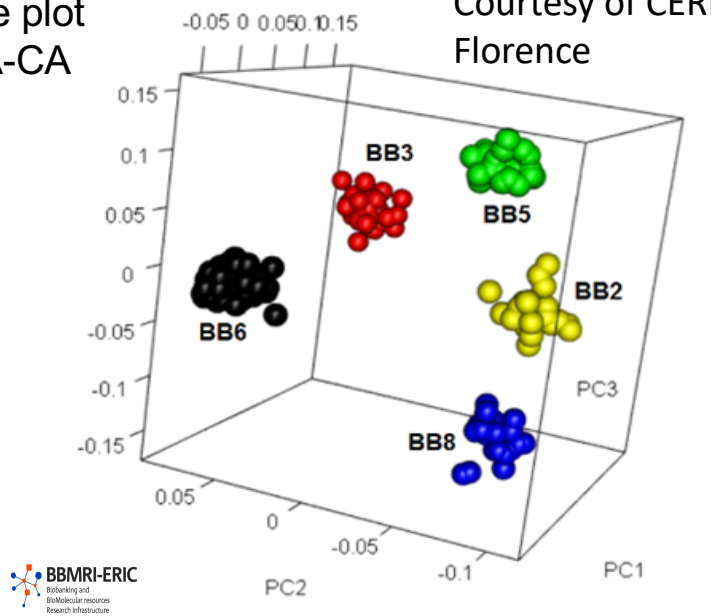
Serum from 5 biobanks

Sample source determins the metabolome signature

Score plot
PCA-CA



Courtesy of CERM-Florence



- Discrimination accuracy = **92%**

Image made available by Kurt Zatloukal

Project in collaboration with BBMRI-ERIC (Biobanking and BioMolecular resources Research Infrastructure – European Research Infrastructure Consortium)

Why pre-analytics?

➢Medical research irreproducibility, which slows down the translation into medical practice

Sources of variability related to clinical research irreproducibility

*#Tissue and macromolecule **pre-analytical preservation** (pre- and fixation procedures)*
*#Selection and **standardization** of analytical procedures (standardization of procedures, controls, interpretation of results)*
***#Heterogeneity** on morphological and molecular level*

The Economist. 2013 Oct How Science goes wrong

Major efforts for improvement

- **Technologies** for securing high quality samples
- **International Standards** for pre-analytical workflows

What is a standard?

It is a reference model to which you may conform.

The standard or norm is a document, used in various areas, which establishes technical specifications for the realization of a product or the provision of a service.

Those documents are created by International normation bodies- CEN and ISO and the National counterparts.

https://youtu.be/XMjQY2QzZ_U?list=PLdF-R_TmJXfgqxLlcUfEml45_Ph_55ECe

Pre-analytical Workflow - Standards for all Segment

◎ **Biobanks**

• Source for high quality samples

BBMRI-ERIC plays a central role

◎ **Biomedical & Translational Research**

• Academia

• Pharma industry

• Diagnostic Industry

◎ **Diagnostics**

• High sample quality is mandatory for reliable diagnostic results

• Analytical assay might tolerate lower quality or not

→Validation studies

ISO 20184-2:2018

Molecular in vitro diagnostic examinations -- Specifications for pre-examination processes for frozen tissue Isolated proteins

ISO 20184-1:2018

Molecular in vitro diagnostic examinations -- Specifications for pre-examination processes for frozen tissue Isolated RNA

ISO 20166-2:2018

Molecular in vitro diagnostic examinations -- Specifications for pre-examinations processes for formalin-fixed and paraffin-embedded (FFPE) tissue Isolated proteins

ISO 20166-1:2018

Molecular in vitro diagnostic examinations -- Specifications for pre-examination processes for formalin-fixed and paraffin-embedded (FFPE) tissue Isolated RNA

ISO 20166-3:2018

Molecular in vitro diagnostic examinations -- Specifications for pre-examination processes for formalin-fixed and paraffin-embedded (FFPE) tissue Isolated DNA

ISO 20186-3:2019

Molecular in vitro diagnostic examinations -- Specifications for pre-examination processes for venous whole blood Isolated circulating cell free DNA from plasma

ISO 20186-1:2019

Molecular in vitro diagnostic examinations -- Specifications for pre-examination processes for venous whole blood Isolated cellular RNA

ISO 20186-2:2019

Molecular in vitro diagnostic examinations -- Specifications for pre-examination processes for venous whole blood Isolated genomic DNA

ISO Technical Specification for FFPE tissues

Contents

International Standards (ISO) and European Technical Specifications (CEN)
BBMRI-ERIC Self-Assessment Survey

AUDITING

If you run a non-certified biobank and/or you want to know if the samples stored fulfill certain quality requirements, get the support of BBMRI.QM. We offer peer-review-style audits on request. Take the next QM improvement step together with us!

Step 1: Assess your processes with the BBMRI-ERIC Self-Assessment Survey (BBMRI-ERIC SAS).

- What is the BBMRI-ERIC SAS?
- Short explanation about access principles to the BBMRI-ERIC SAS
- Request the BBMRI-ERIC SAS

Step 2: Request a BBMRI-ERIC audit

- BBMRI-ERIC Audit

Step 3: A positive audit will lead to a quality mark in the Directory

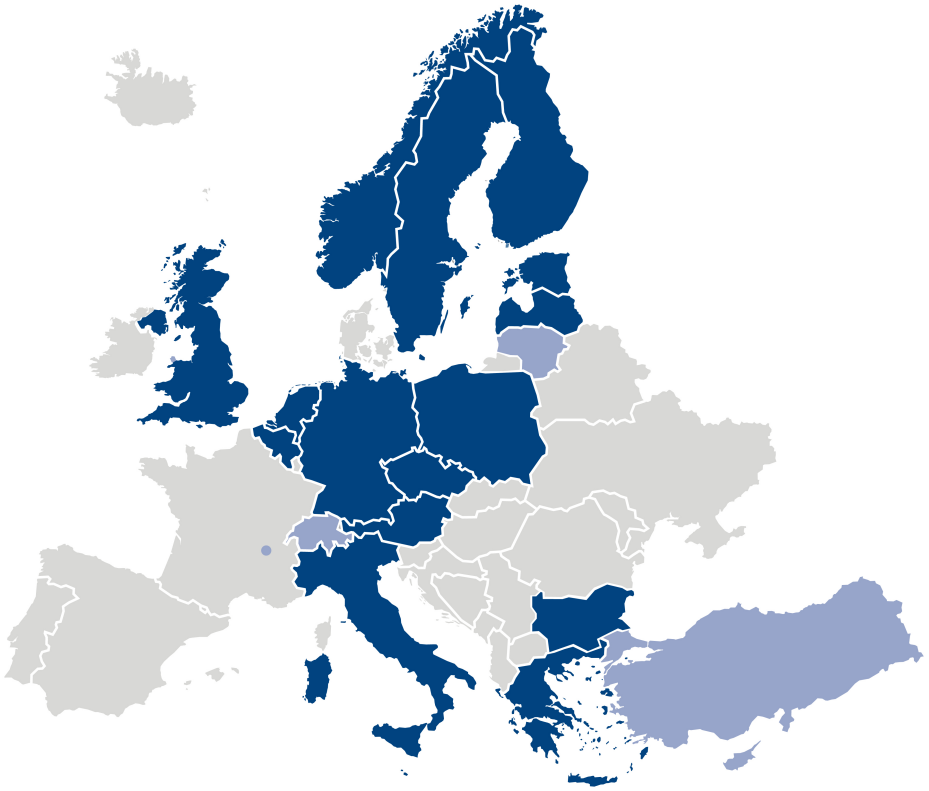
- Q-mark in the BBMRI-ERIC Directory

www.bbmri-eric.eu/services/quality-management/

BBMRI-ERIC Work Programme 2016-2020
CEN/TC 140 and ISO/TC 212
Molecular in vitro diagnostic examinations – Specifications for pre-examination processes

Representatives of Quality Experts Groups:

Austria: 18
Belgium: 19
Bulgaria: 1
Switzerland: 4
Cyprus: 3
Czech Republic: 3
Germany: 15
Estonia: 3
Finland: 20
Greece: 1
IARC: 2
Italy: 10
Latvia: 3
Lithuania: 1
Malta: 6
Netherlands: 4
Norway: 6
Poland: 10
Sweden: 6
Turkey: 12
UK: 1



BBMRI-ERIC Self-Assessment Survey

www.bbmri-eric.eu/services/quality-management/

ACCESS TO BBMRI-ERIC SAS

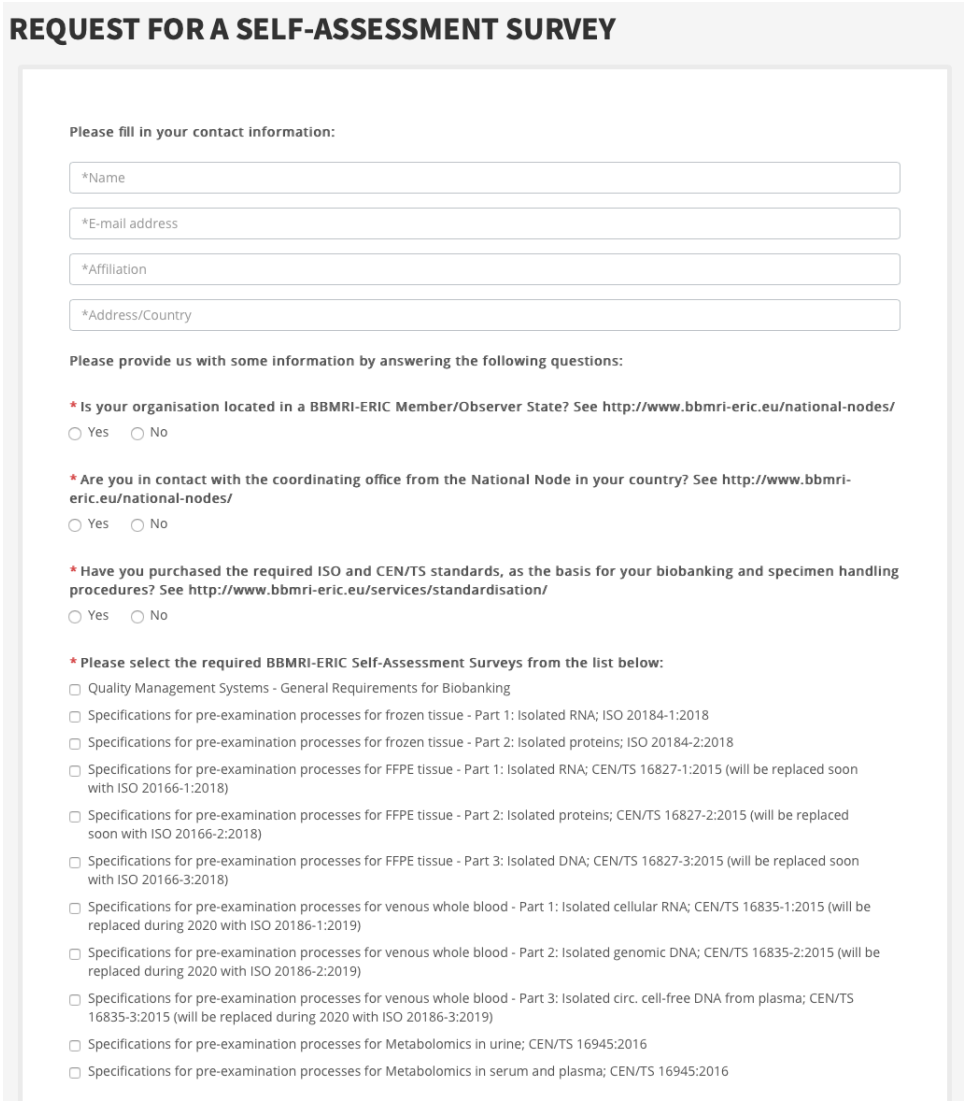
- **GO TO**
bbmri-eric.eu/services/self-assessment-survey/
- **FILL OUT**
Request form / tick off pre-conditions / send
- **GET STARTED**
Receive @ with the link to SAS

EVALUATION OF SPECIFICATIONS

- **COMPLETION**
of BBMRI-ERIC SAS
- **SUBMIT REPORT**
to BBMRI-ERIC
- **BE REVIEWED**
by BBMRI-ERIC (remote or on-site)

AWARD Q-LABEL IN BBMRI-ERIC DIRECTORY

- **SAMPLE COLLECTION**
Assessed according to relevant standards
- **BIOBANK**
Internal audit based on ISO 20387 and ISO 9001
- **ENHANCE VISIBILITY**
Q-Label in the Directory directory.bbmri-eric.eu



REQUEST FOR A SELF-ASSESSMENT SURVEY

Please fill in your contact information:

*Name

*E-mail address

*Affiliation

*Address/Country

Please provide us with some information by answering the following questions:

*** Is your organisation located in a BBMRI-ERIC Member/Observer State? See http://www.bbmri-eric.eu/national-nodes/**

○ Yes ○ No

*** Are you in contact with the coordinating office from the National Node in your country? See http://www.bbmri-eric.eu/national-nodes/**

○ Yes ○ No

*** Have you purchased the required ISO and CEN/TS standards, as the basis for your biobanking and specimen handling procedures? See http://www.bbmri-eric.eu/services/standardisation/**

○ Yes ○ No

*** Please select the required BBMRI-ERIC Self-Assessment Surveys from the list below:**

- ☐ Quality Management Systems - General Requirements for Biobanking
- ☐ Specifications for pre-examination processes for frozen tissue - Part 1: Isolated RNA; ISO 20184-1:2018
- ☐ Specifications for pre-examination processes for frozen tissue - Part 2: Isolated proteins; ISO 20184-2:2018
- ☐ Specifications for pre-examination processes for FFPE tissue - Part 1: Isolated RNA; CEN/TS 16827-1:2015 (will be replaced soon with ISO 20166-1:2018)
- ☐ Specifications for pre-examination processes for FFPE tissue - Part 2: Isolated proteins; CEN/TS 16827-2:2015 (will be replaced soon with ISO 20166-2:2018)
- ☐ Specifications for pre-examination processes for FFPE tissue - Part 3: Isolated DNA; CEN/TS 16827-3:2015 (will be replaced soon with ISO 20166-3:2018)
- ☐ Specifications for pre-examination processes for venous whole blood - Part 1: Isolated cellular RNA; CEN/TS 16835-1:2015 (will be replaced during 2020 with ISO 20186-1:2019)
- ☐ Specifications for pre-examination processes for venous whole blood - Part 2: Isolated genomic DNA; CEN/TS 16835-2:2015 (will be replaced during 2020 with ISO 20186-2:2019)
- ☐ Specifications for pre-examination processes for venous whole blood - Part 3: Isolated circ. cell-free DNA from plasma; CEN/TS 16835-3:2015 (will be replaced during 2020 with ISO 20186-3:2019)
- ☐ Specifications for pre-examination processes for Metabolomics in urine; CEN/TS 16945:2016
- ☐ Specifications for pre-examination processes for Metabolomics in serum and plasma; CEN/TS 16945:2016

BBMRI-ERIC Self-Assessment Survey

SPIDIA for personalised medicine: Standardisation and improvement of generic pre-analytical tools and procedures for in-vitro diagnostics

- ✓ 48-month project
- ✓ key experts of 19 stakeholder organisations
- ✓ Aims: pre-analytical procedures, European and international standardisation organisations' processes (CEN and ISO), external quality assurance, quality management, ethics and regulatory demands
- ✓ www.spidia.eu

CEN Technical Specifications for Pre-examination Processes

Development of 12 new CEN/TS and 2 ISO standards & Raising awareness for and implementation of standards

4 Venous whole blood circul. tumor cells — RNA, DNA, protein & staining procedures
1 Venous whole blood exosomes — cfc RNA
1 Frozen tissue — DNA
1 Urine/other body fluids - cfcDNA
3 fine needle aspirates – RNA, DNA, protein
1 Saliva & stool microbiomes– DNA
1 Saliva — DNA

CEN/TS

1 FFPE tissue – in-situ staining
1 Metabolomics – urine, plasma, serum

ISO

13 new External Quality Assurance Schemes corresponding to the pre-analytical standards portfolio

- ✓ Venous Whole Blood: Genomic DNA and cellular RNA, viable PBMC, Cell Free Circulating DNA(ccfDNA), Cell Free Circulating RNA (ccfRNA), Circulating Tumour Cells (CTCs)
- ✓ FFPE tissue : DNA, RNA, protein
- ✓ Frozen tissue: Genomic DNA, RNA, protein
- ✓ Saliva: DNA
- ✓ Stool: DNA