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DEGLI STUDI DI TRIESTE

OECI
Organisation of European Cancer Institutes

BBMRI-ERIC®
Biobanking and
BioMolecular resources
Research Infrastructure

SPIDIA4P

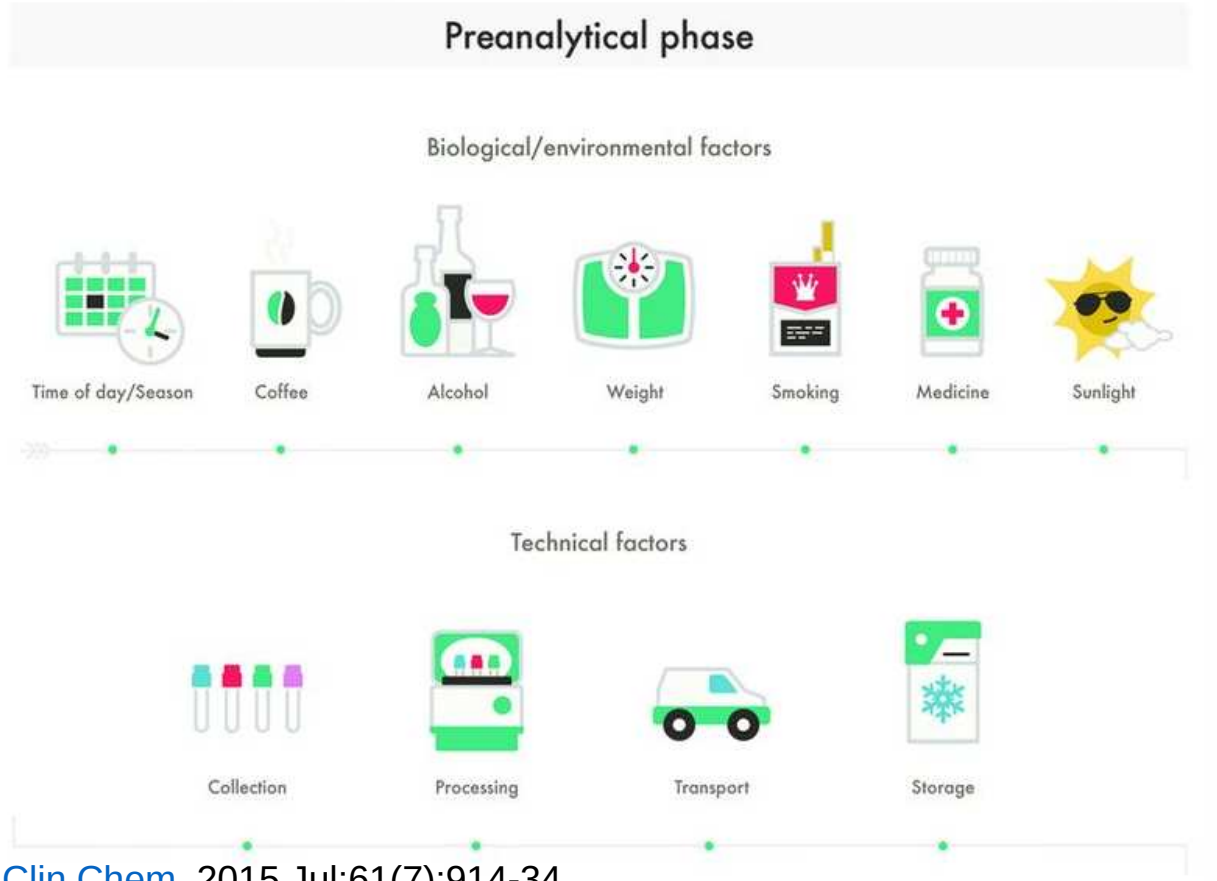
OECI Oncology Days
Brno, 21st - 23rd June 2017
Pathology Day

SPIDIA4P: PREANALYTICAL CONDITIONS OF TISSUES

Serena Bonin
Deparment of Medical Sciences
Università degli Studi di Trieste

What is pre-analytics?

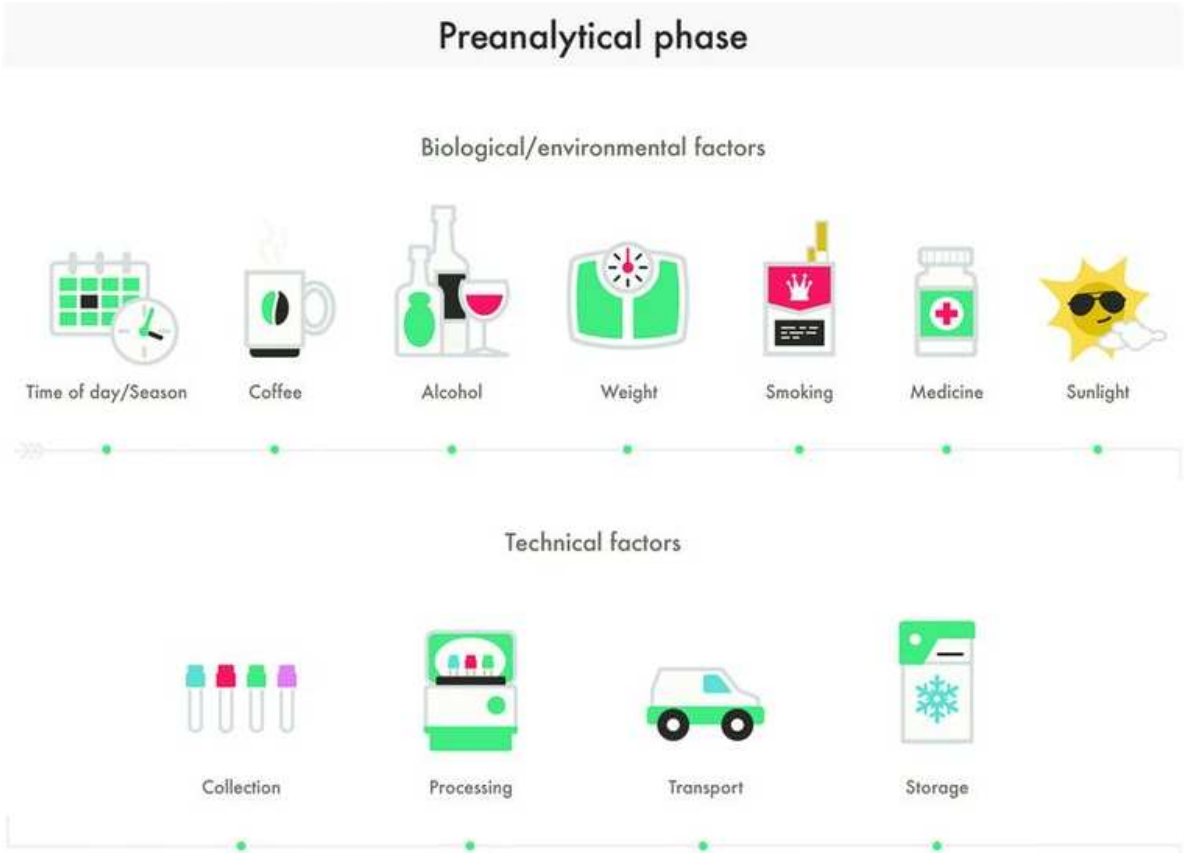
Pre-analytical phase: covers all steps from the clinicians requests to the beginning of the analytical examination, included nucleic acid or protein extractions



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

Why extractions into pre-analytics?

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



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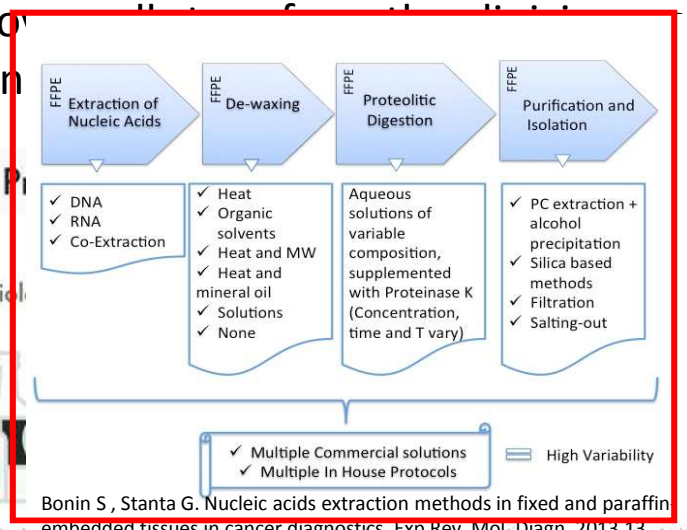
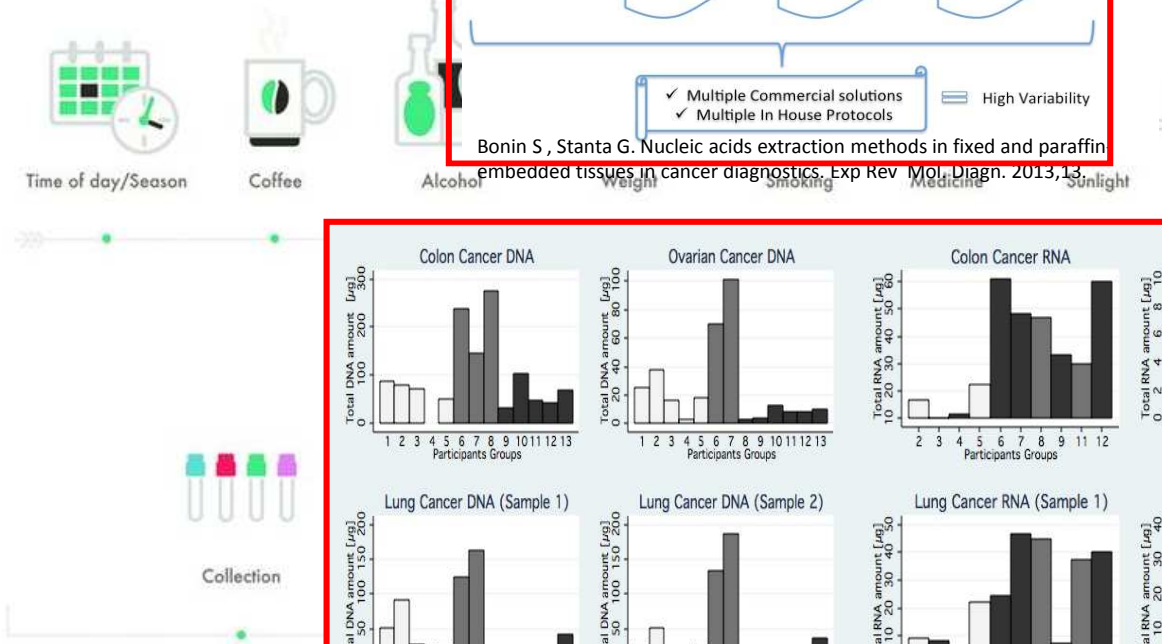
Why extractions into pre-analytics?

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.



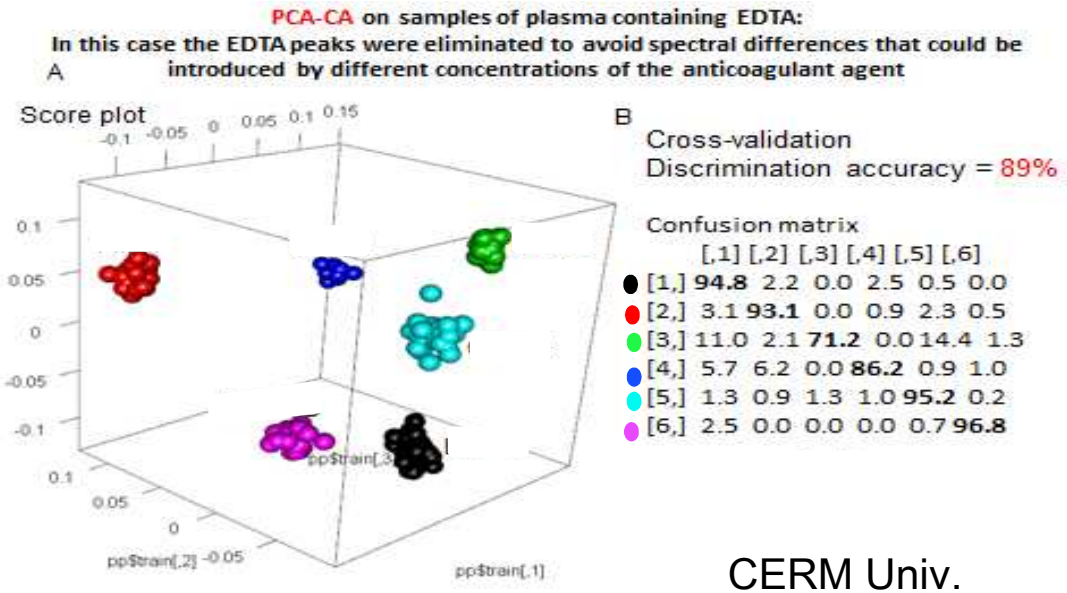
[Clin Chem.](#) 2015 Ju…

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

Sample source determins the metabolome signature

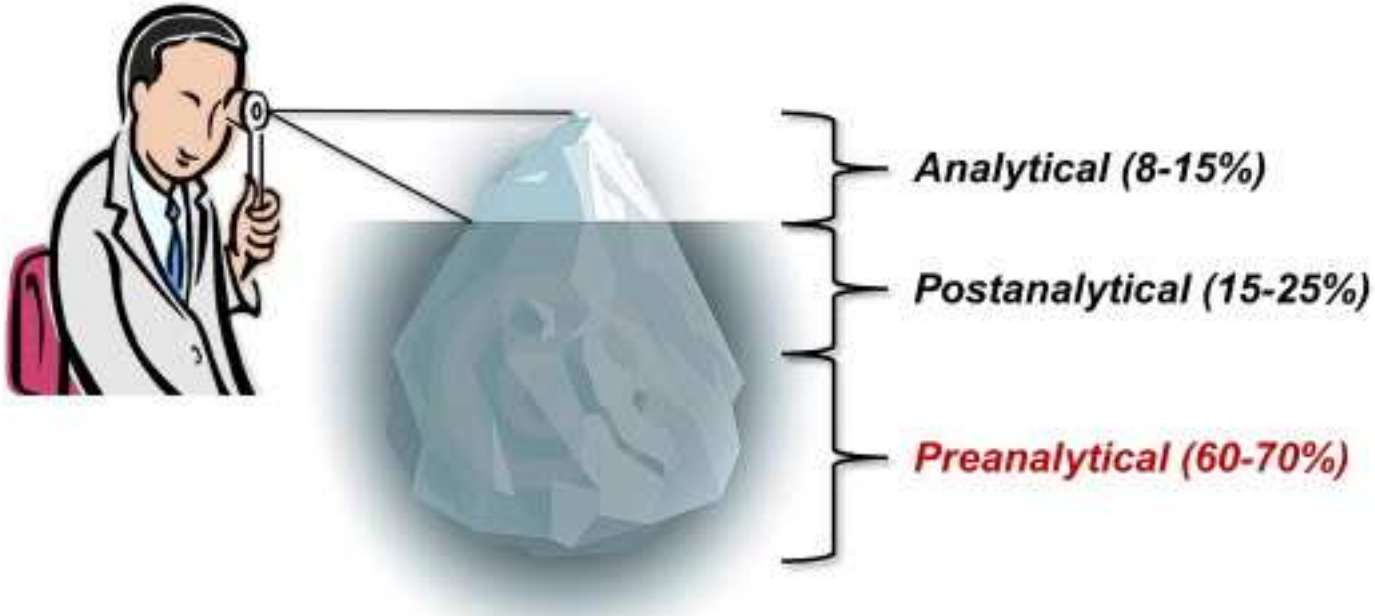


CERM Univ.
Florence

Image made available by Kurt Zatloukal

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

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

Why pre-analytics? Why in FFPE?

SPIDIA

> Formalin fixation and paraffin embedding are part of a globally applied method of tissue preservation; however, they also represent a multistage process that is far from standardized. A recent review article[1] published by our office identified 15 preanalytical factors associated with formalin fixation and paraffin embedding tissue processing that have documented effects on immunohistochemistry (IHC) efficacy and many more that were unaddressed or underaddressed in the scientific literature. While technological

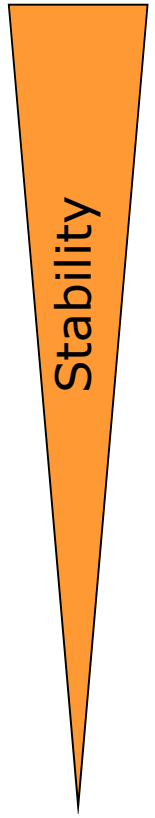
Arch Pathol Lab Med—Vol 138, November 2014

Sample variables

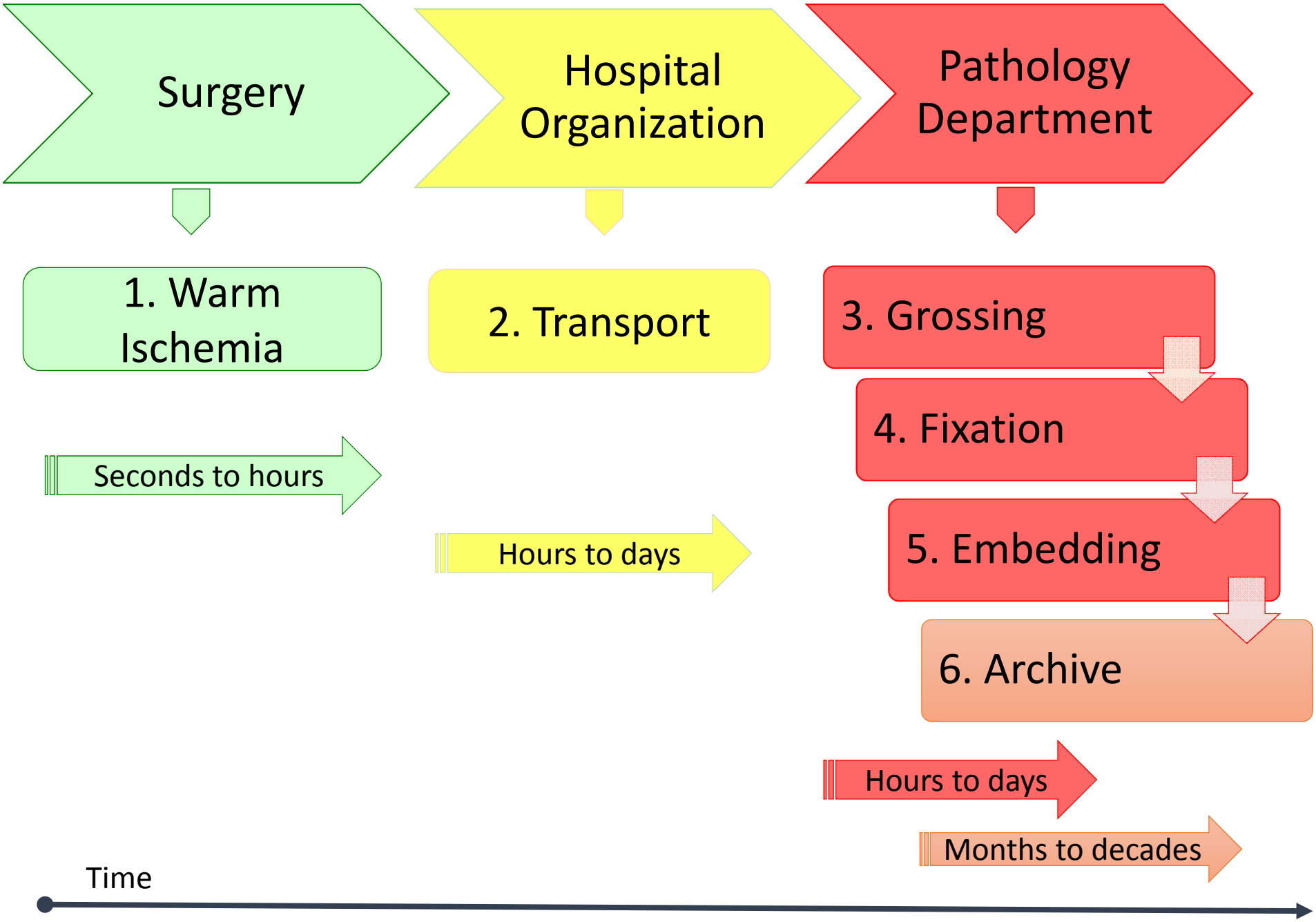
- Tissue type (organ)
- Diseased/normal
- Sample type (biopsy/surgery)
- Peri-operative effects
- Ischemia
- Processing
- Fixation
- Storage
- Analysis

Readout

- Morphology
- Antigenicity
- Mol.structure
- Biomolecules
  - DNA
  - Protein
  - Protein mod.
  - RNA
  - Metabolites
- Interactomes



Original design made available by Kurt Zatloukal


Surgery
Hospital Organization
Pathology Department
1. Warm Ischemia
2. Transport
3. Grossing
4. Fixation
5. Embedding
6. Archive
Seconds to hours
Hours to days
Hours to days
Months to decades
Time

SPIDIA

IMPACTS

can be avoided

can be reduced

not avoidable

Medication
Surgical procedure
Warm ischemia

Transport
Temperature
Cold ischemia

Sample processing
Mech. alteration
Selection+annotation

Aliquotting

Freezing
Freezing rate
Temperature

Cryostorage
Temperature
Temp. shifts

Fixation
Fixative
Time

Embedding
Temperature

Diagnosis
Disease codes

Storage
Time
temperature

Sample
preparation

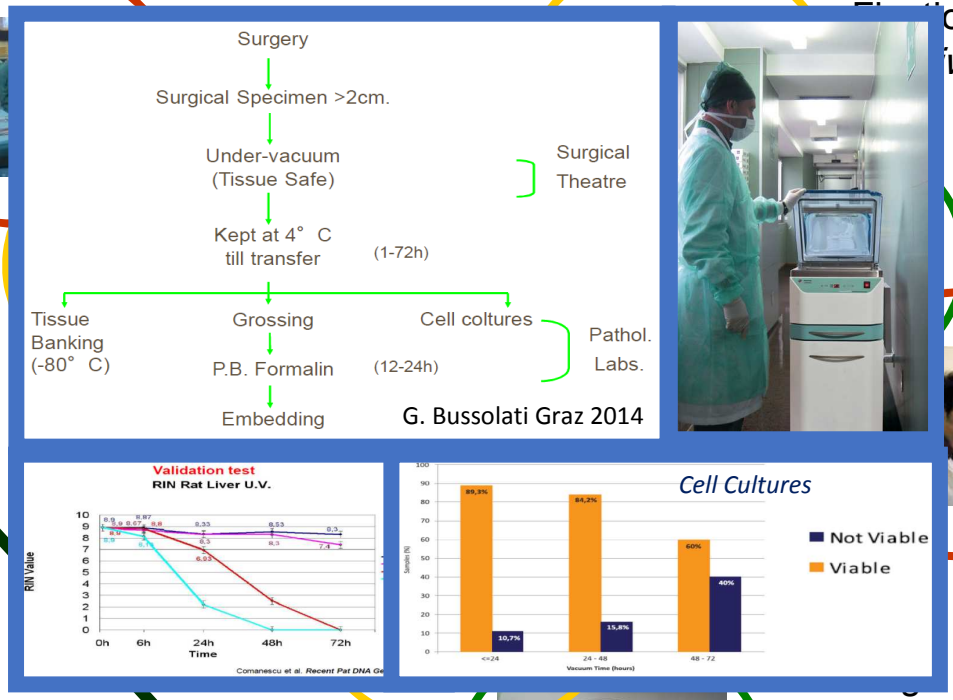
Analysis

Original design made available by Kurt Zatloukal

PREANALYTICAL CONDITIONS

SURGICAL TISSUES

Vacuum system



Cold fixation and
defined time of fixation can be avoided

be reduced

avoidable

RNA TO 600 BASES

Freezing rate
Temperature

Sample
preparation

Cryostorage
Temperature
Temp. shifts

Analysis

Original design made available by Kurt Zatloukal

SPIDIA→ 9 CEN/TS- European Technical Specification

Molecular in-vitro diagnostic examinations - Specifications for pre-examination processes for:

- **blood**: cellular RNA –CEN/TS 1865-1
- **blood**: genomic DNA-CEN/TS 1865-2
- **blood**: cell free circulating DNA -CEN/TS 1865-3
- **FFPE tissue**: RNA - CEN/TS 16827-1
- **FFPE tissue**: Proteins- CEN/TS 16827-2
- **FFPE tissue**: DNA- CEN/TS 16827-3
- **snap frozen tissue**: RNA CEN/TS 16826-1
- **snap frozen tissue**: Proteins CEN/TS 16826-1
- **metabolomics** in urine, serum and plasma CEN/TS 16945

CEN/TS- European Technical Specification

Target groups

- ✓ *In-vitro* diagnostic laboratories
- ✓ *In-vitro* diagnostics developers and manufacturers
- ✓ Institutions and commercial organizations performing biomedical and clinical research
- ✓ Biobanks
- ✓ Regulation authorities

CEN/TS- European Technical Specification

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BBMRI-ERIC Self assessment Survey

BBMRI-ERIC website

Standards and best practices for biobanking recommended

- *Standardisation*
- *"Quality Management Services" Flyer*

Sharing QM expertise on a European scale

- *See QM info & experts!*

Self Assessment Surveys

- *Access*

BBMRI-ERIC Work Programme 2016

Quality Work Stream 2.1 CEN/TC 140 / ISO 212 Quality of the sample

BBMRI-ERIC
Biobanking and BioMolecular Resources Research Infrastructure

Representatives of the BBMRI-ERIC Quality Expert Working Groups from 18 different countries

European Committee for Standardization

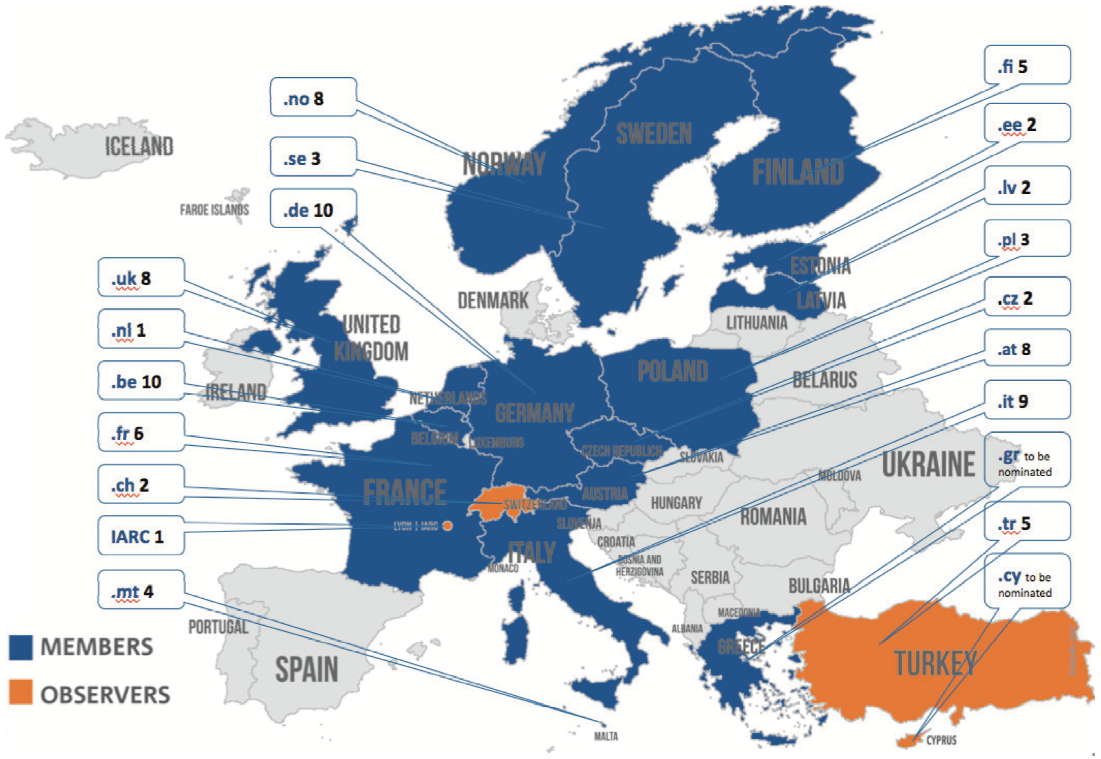


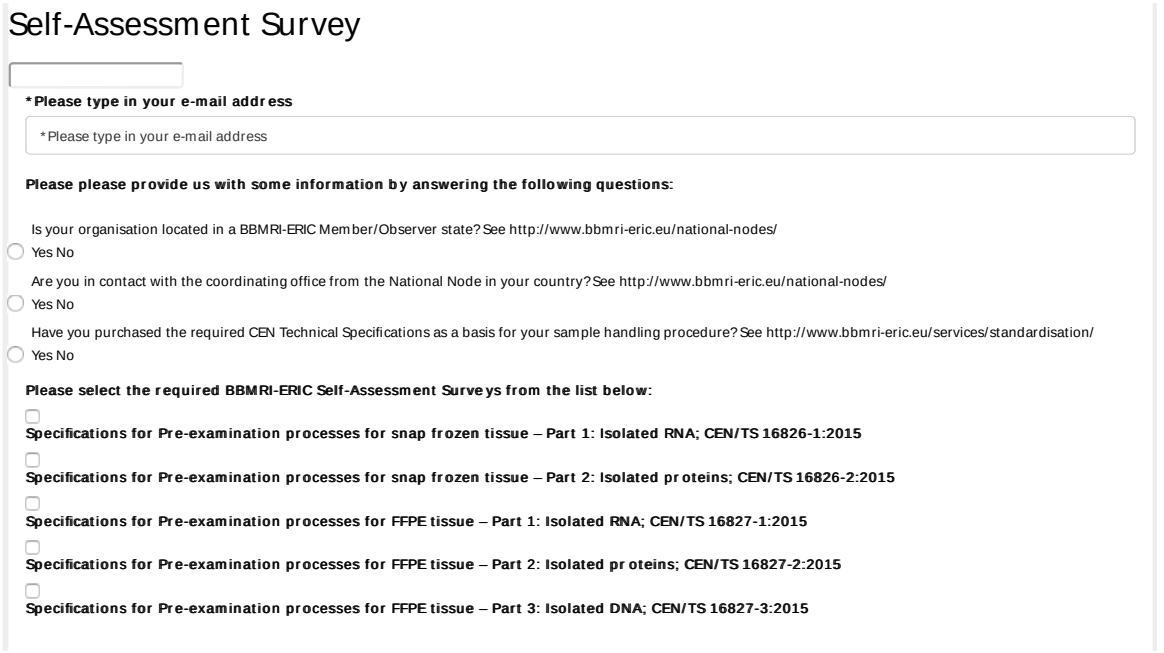
Image made available by Giorgio Stanta

BBMRI-ERIC Self assessment Survey

Registration

Compliance Assessment

Model 1: Biobank internal use



Self-Assessment Survey

*Please type in your e-mail address

*Please type in your e-mail address

Please 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 CEN Technical Specifications as a basis for your sample handling procedure? See http://www.bbmri-eric.eu/services/standardisation/

Yes No

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

Specifications for Pre-examination processes for snap frozen tissue – Part 1: Isolated RNA; CEN/TS 16826-1:2015

Specifications for Pre-examination processes for snap frozen tissue – Part 2: Isolated proteins; CEN/TS 16826-2:2015

Specifications for Pre-examination processes for FFPE tissue – Part 1: Isolated RNA; CEN/TS 16827-1:2015

Specifications for Pre-examination processes for FFPE tissue – Part 2: Isolated proteins; CEN/TS 16827-2:2015

Specifications for Pre-examination processes for FFPE tissue – Part 3: Isolated DNA; CEN/TS 16827-3:2015

Model 2: Biobank submits report to BBRI-ERIC

BBMRI-ERIC grading- biobank and samples signed as compliant to the specific CEN/TS

BBMRI-ERIC Self assessment Survey

Primary tissue collection

> Information about the sample donor

12)	**Donor/patient ID documented should>**	Yes / No / reset e.g. code
13)	**Health status of donor/patient documented should**	Yes / No / reset e.g. healthy, disease type, concomitant disease
14)	**Medical treatment documented should**	Yes / No / reset e.g. anaesthetics, medications, surgical or diagnostic procedures
	Start of warm ischemia documented: **********	
15)	**Date of vessel ligation/arterial clamping time should**	Yes / No / reset
16)	**Time of vessel ligation/arterial clamping time should**	Yes / No / reset

Information on the primary tissue sample

	Start of cold ischemia documented: **********	
17)	**Date of tissue removal from the body shall**	Yes / No / reset
18)	**Time of tissue removal from the body shall**	Yes / No / reset
	Tissue type and condition documented: **********	
19)	**General tissue type and condition shall**	Yes / No / reset
20)	**Organ of origin and location within shall**	Yes / No / reset
	Type and start of fixation documented: ********** **(if started outside the biobank)**	
21)	**Date of start shall**	Yes / No / reset
22)	**Time of start shall**	Yes / No / reset
23)	**Fixative type shall**	Yes / No / reset
24)	**Fixative condition shall**	Yes / No / reset

Information on the primary tissue sample processing

25)	**Modifications after removal from body documented shall**	Yes / No / reset e.g. labelling for specimen orientation such as ink-marking, stitches, incisions
26)	**Selection/use of transport containers performed shall**	Yes / No / reset e.g.cooling box, vaccum packing
27)	**Selection/use of stabilisation procedures for transport of unfixed primary tissue performed shall**	Yes / No / reset e.g.cooling methods, fixation
28)	**Labelling of the transport container performed shall**	Yes / No / reset e.g.registration number, barcode (1D ord 2D), primary sample type, quantity, organ origin of tissue
29)	**Documented, when several aliquots of a single sample with different features are in one container shall**	Yes / No / reset

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

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CEN

- RNA - CEN/TS 16827-1
- Proteins- CEN/TS 16827-2
- DNA- CEN/TS 16827-3

ISO/TC 212

- ISO/DIS 20166-1, -2,-3: Molecular in vitro diagnostic examinations: Specifications for pre-examination processes for FFPE tissues (1-RNA, 2-protein, 3-DNA)

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CEN

- RNA CEN/TS 16826-1
- Proteins CEN/TS 16826-1

ISO/TC 212

- ISO/DIS 20184-1, -2,: Molecular in vitro diagnostic examinations: Specifications for pre-examination processes for frozen tissues (1-RNA, 2-protein)

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CEN

- cellular RNA – CEN/TS 1865-1
- genomic DNA- CEN/TS 1865-2
- cell free circulating DNA - CEN/TS 1865-3

ISO/TC 212

- ISO/DIS 20184-1, -2,: Molecular in vitro diagnostic examinations: Specifications for pre-examination processes for venous whole blood(1-RNA, 2-gDNA, 3-cfDNA from plasma)

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

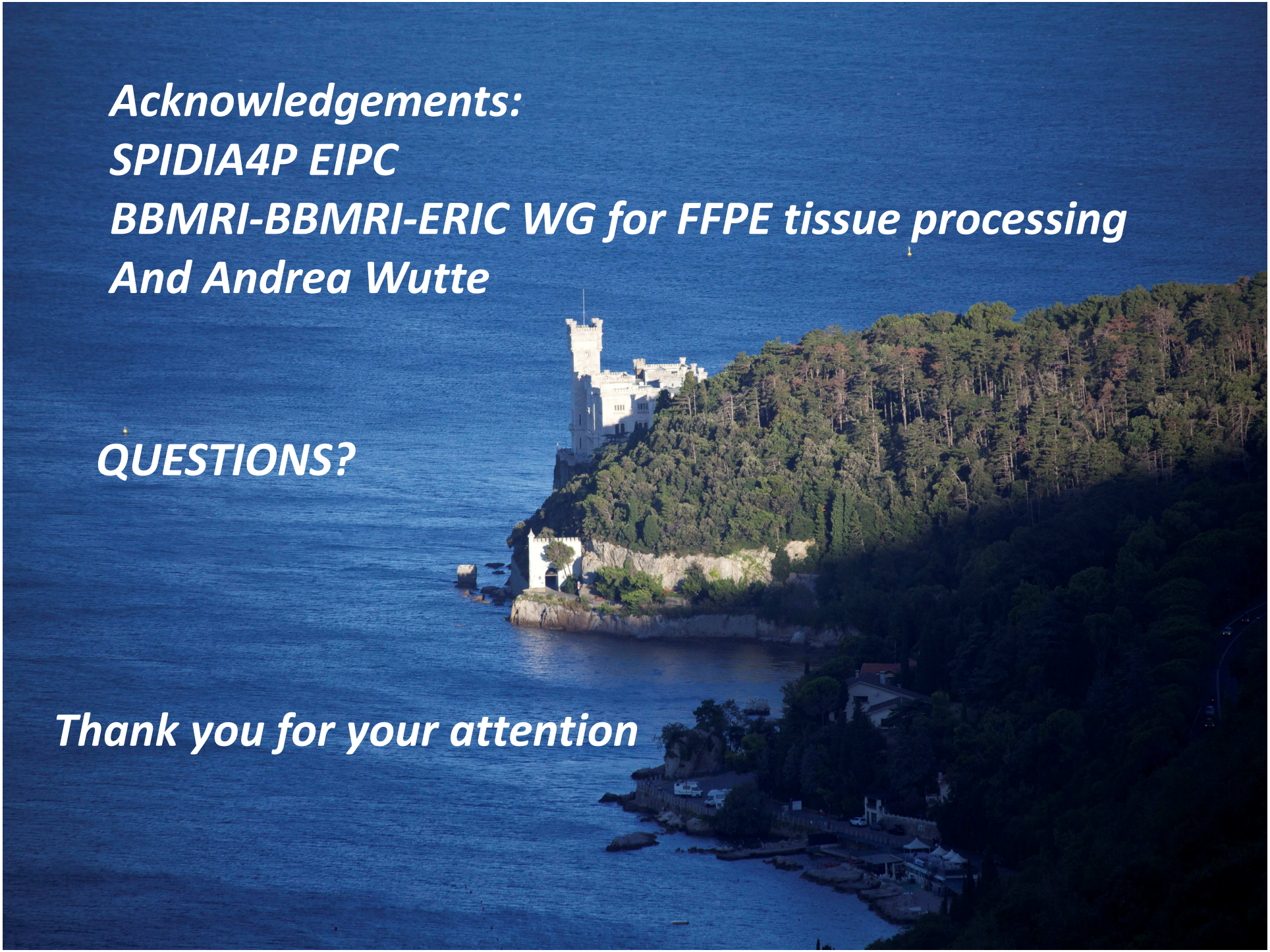
CEN/TS

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



Acknowledgements:
SPIDIA4P EIPC
BBMRI-BBMRI-ERIC WG for FFPE tissue processing
And Andrea Wutte

QUESTIONS?

Thank you for your attention