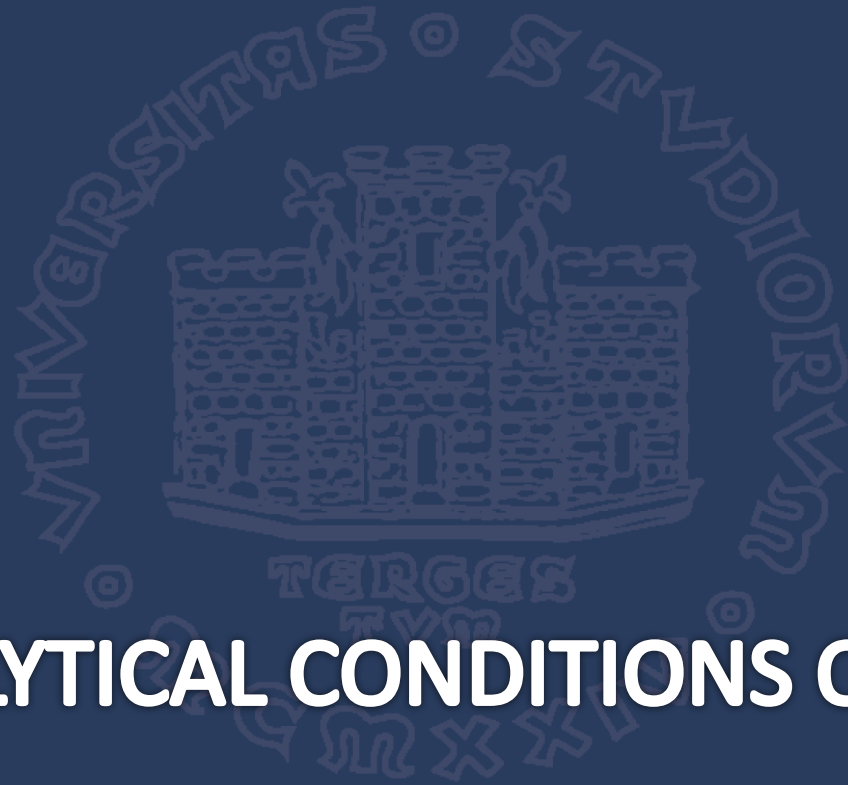




UNIVERSITÀ  
DEGLI STUDI DI TRIESTE



# PREANALYTICAL CONDITIONS OF TISSUES

*Serena Bonin*

Department of Medical Sciences  
Università degli Studi di Trieste

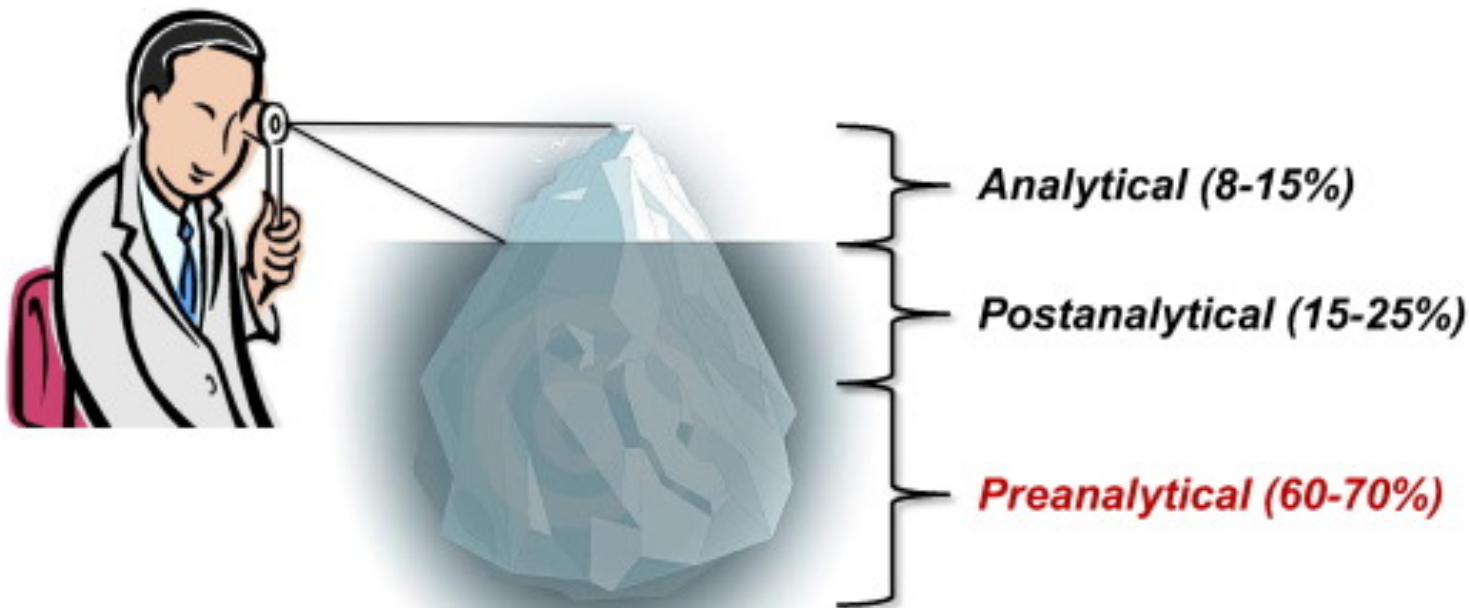


# Why pre-analytics?

➤ 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

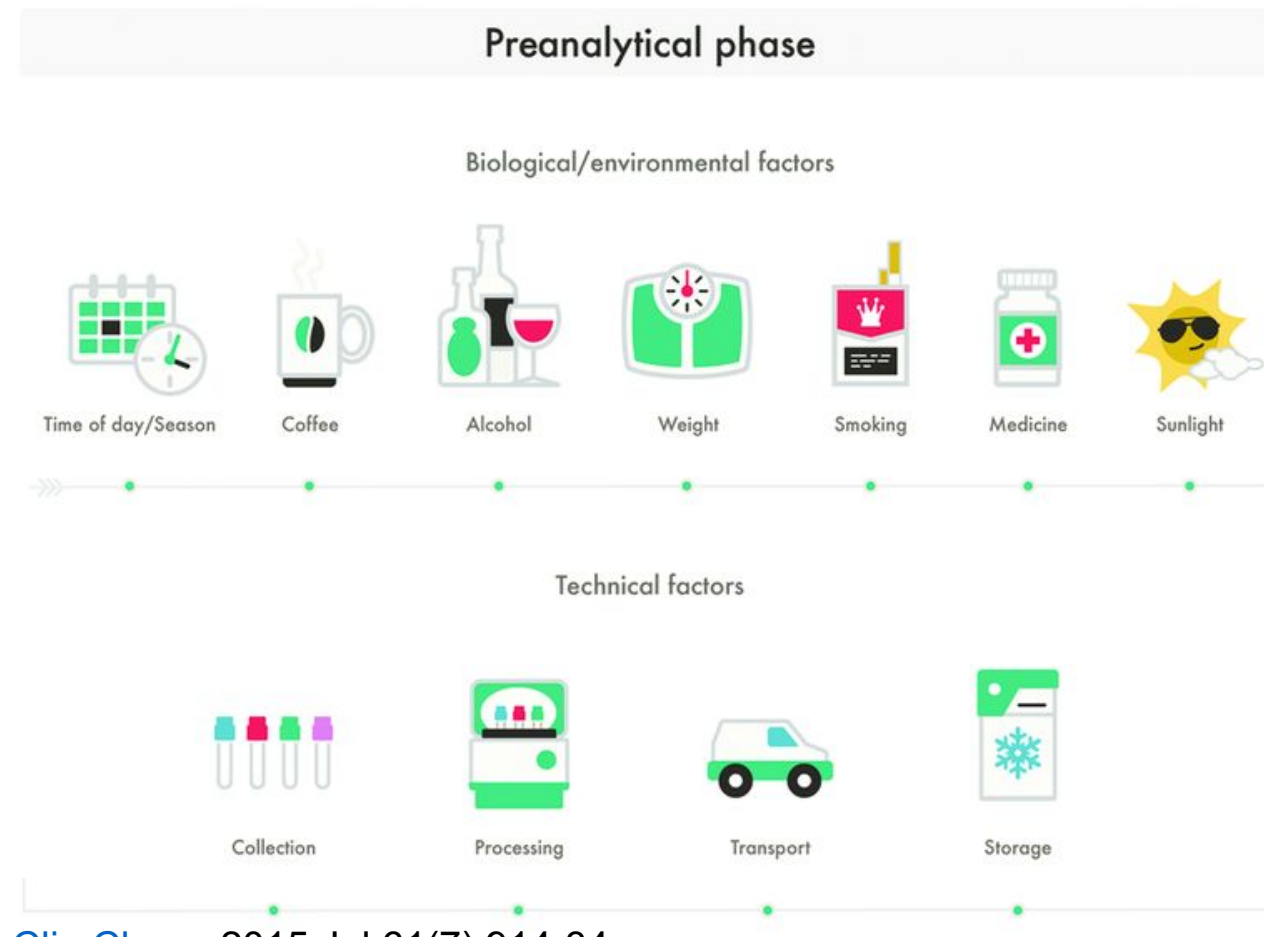
May;46(5):22, 24, 26)

➤ **10<sup>7</sup> €** 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)



# 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

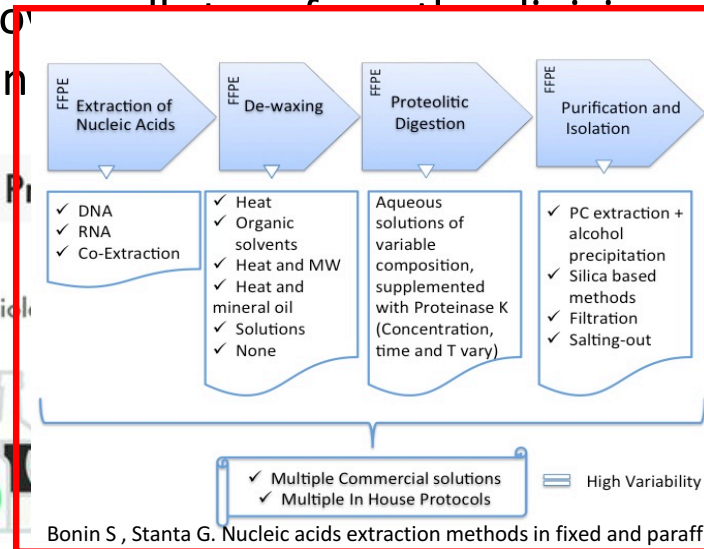


[Clin Chem.](#) 2015 Jul;61(7):914-34

# Why extractions into pre-analytics?

Pre-analytical phase: core of the analytical examination

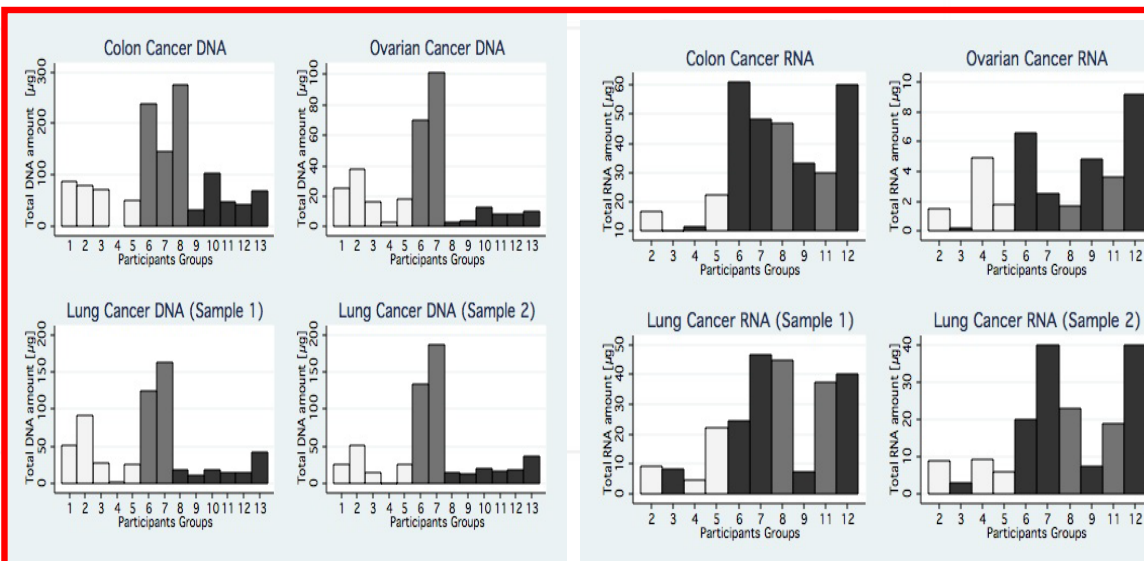
requests to the beginning in extractions



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 Jun



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

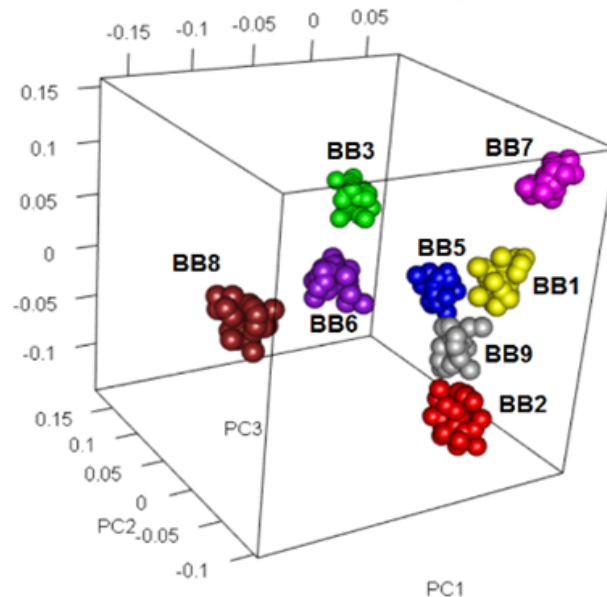


# Why pre-analytics?

- 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

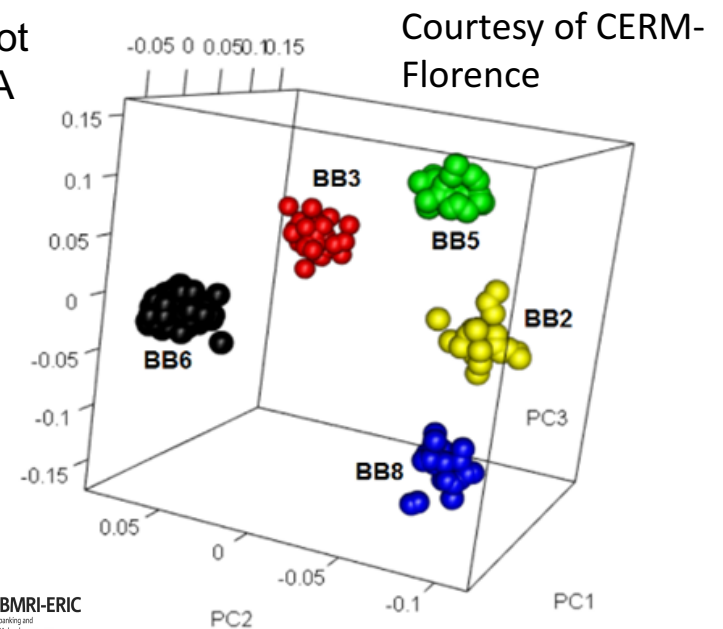
European healthy subjects

**EDTA-plasma** from 9 biobanks



**Serum** from 5 biobanks

Score plot  
PCA-CA



Courtesy of CERM-Florence

❖ Discrimination accuracy = **92%**



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

Sample source  
determines the  
metabolome  
signature

# 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

# *AIMS OF PREANALYTICAL CONDITIONS*

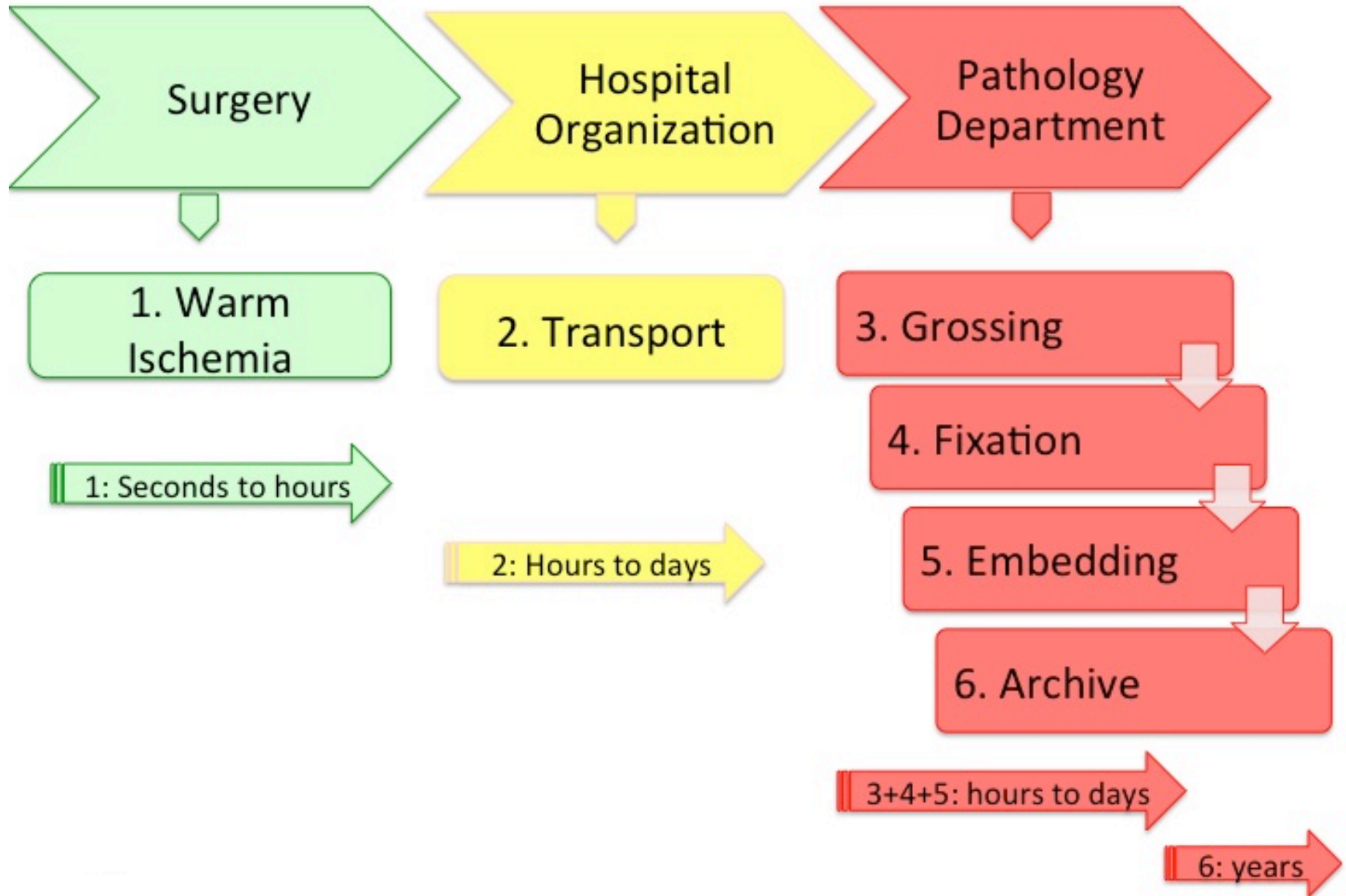
## *Preservation in Archive FFPE Tissues of*

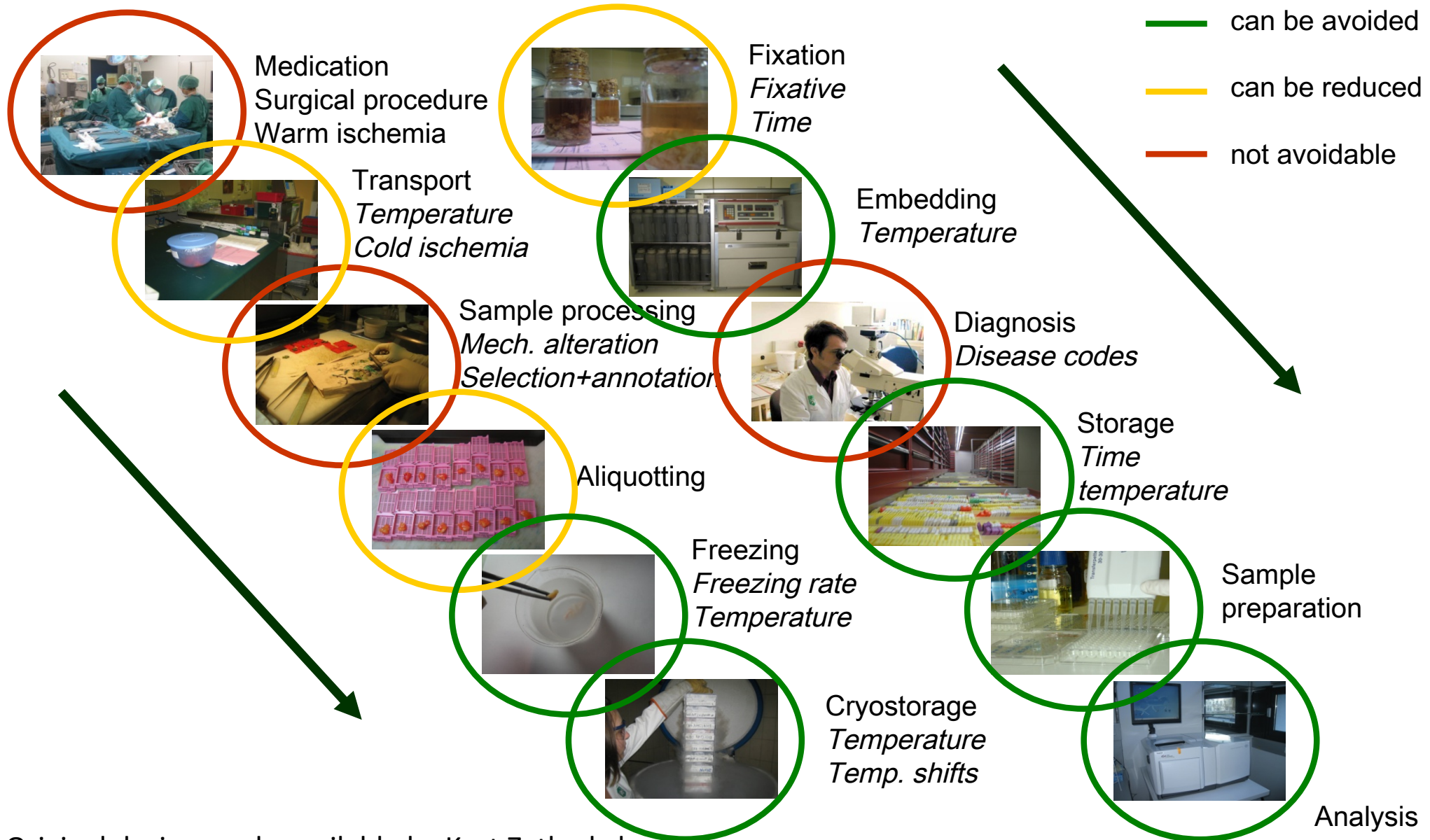
- *Structure (morphological diagnosis)*
- *Proteins (Immunohistochemistry +Extraction)*
- *Nucleic Acids ( ISH + Extraction )*

# ***Preamanalytical Conditions***

**Outside the pathology lab**

**Inside the pathology lab**



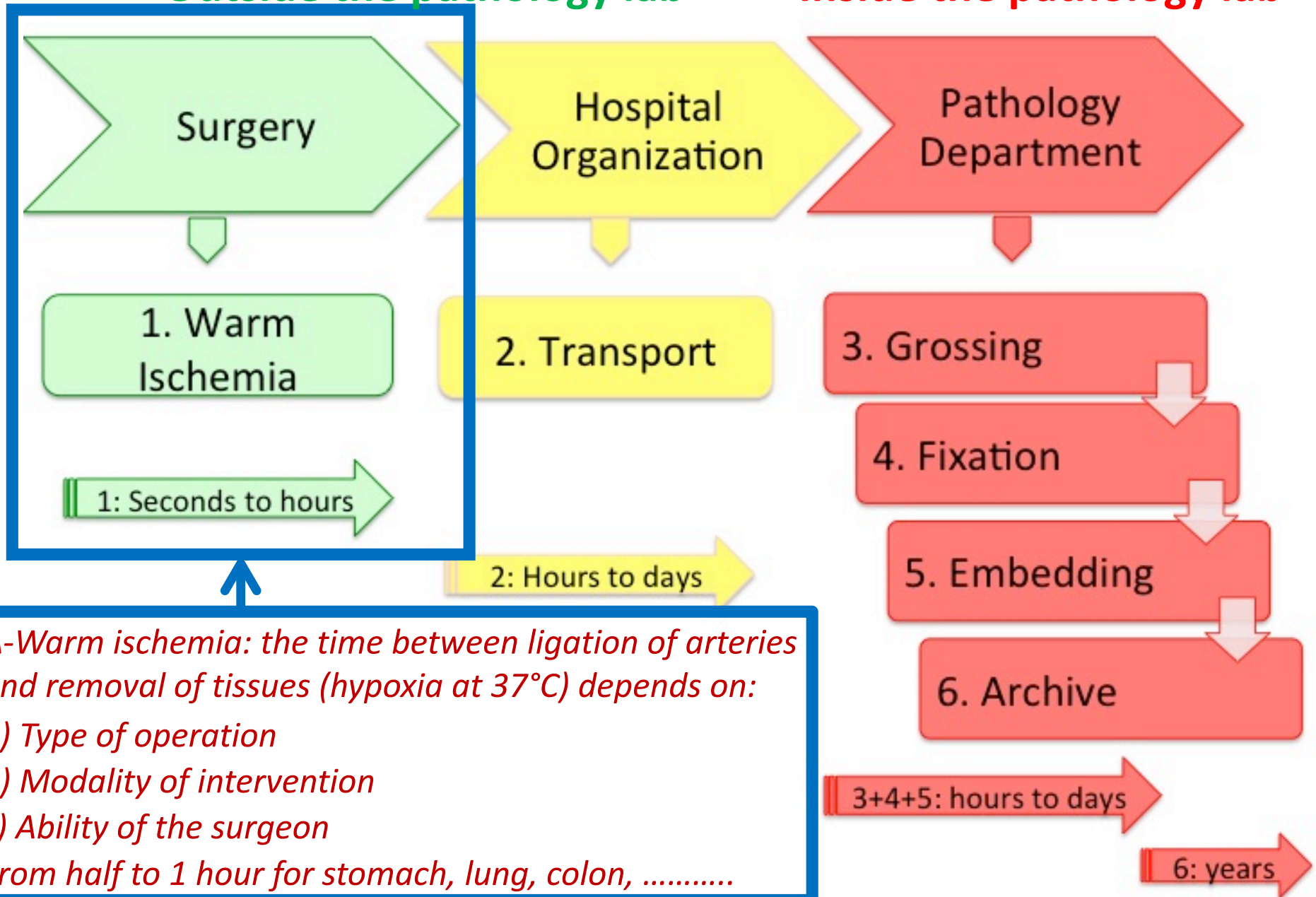




# Preamanalytical Conditions

Outside the pathology lab

Inside the pathology lab

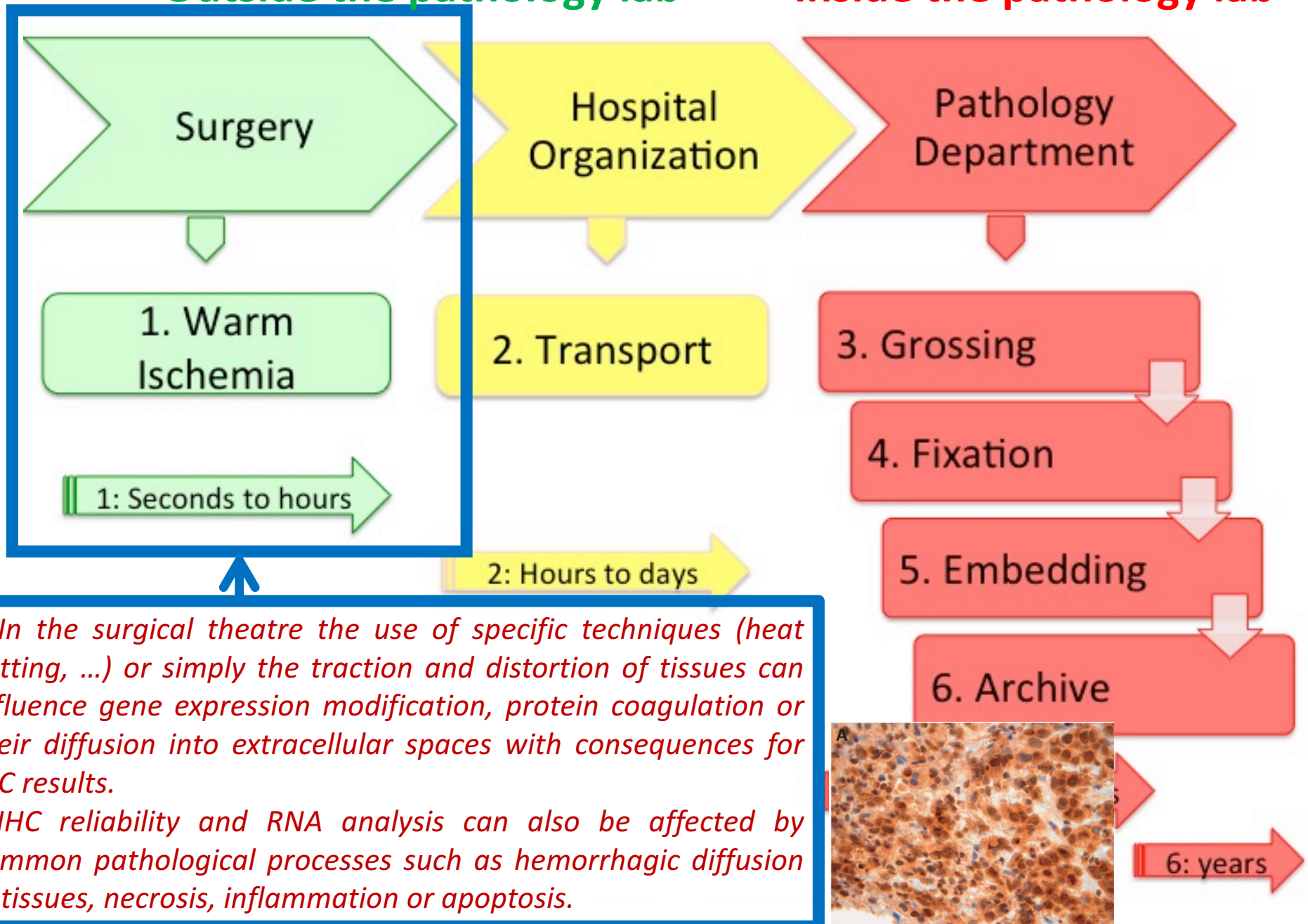




# Pre-analytical Conditions

Outside the pathology lab

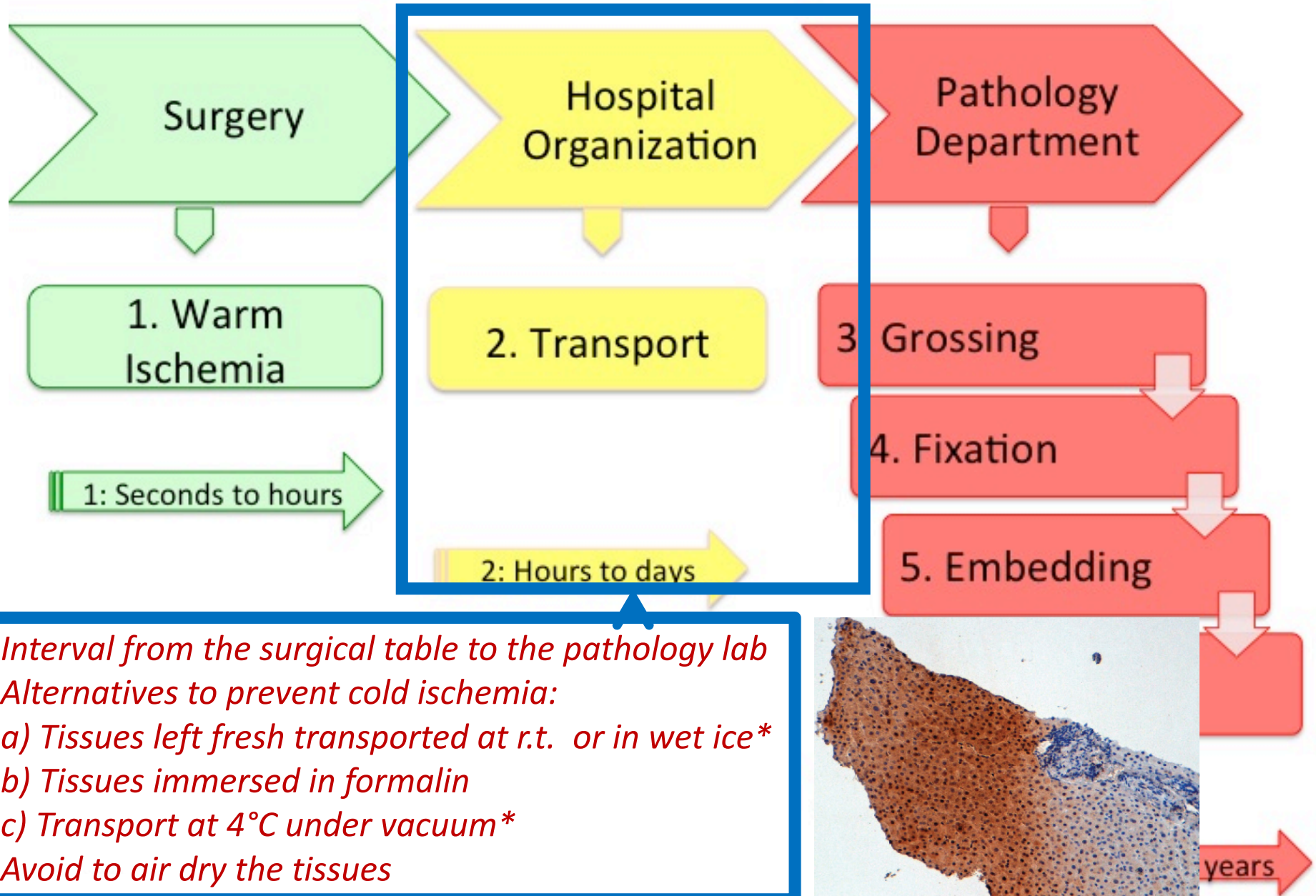
Inside the pathology lab



# ***Pre-analytical Conditions***

**Outside the pathology lab**

**Inside the pathology lab**



*Interval from the surgical table to the pathology lab  
Alternatives to prevent cold ischemia:*

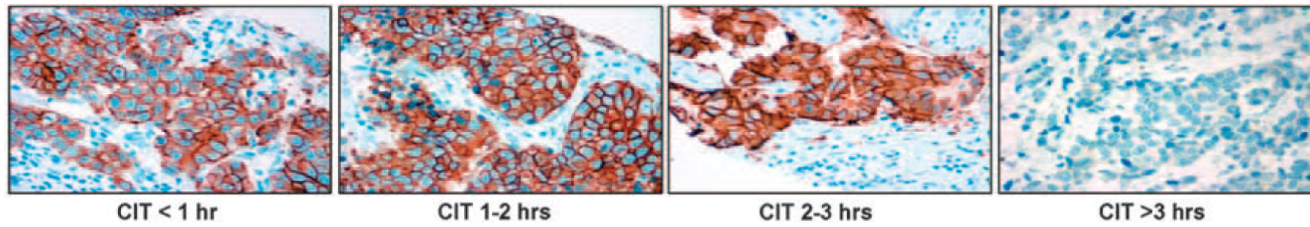
- a) Tissues left fresh transported at r.t. or in wet ice\**
- b) Tissues immersed in formalin*
- c) Transport at 4°C under vacuum\**

*Avoid to air dry the tissues*

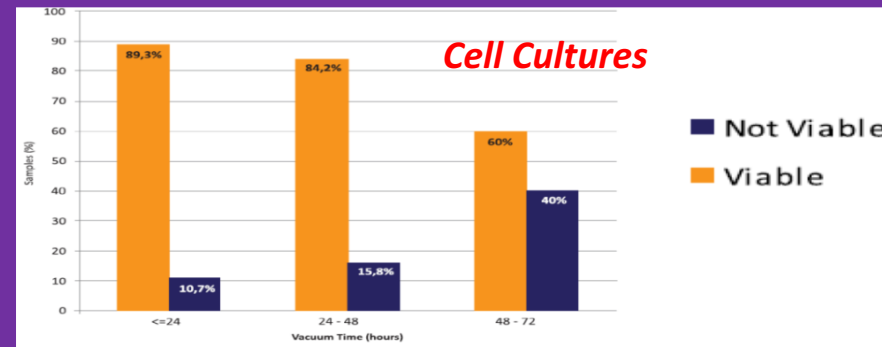
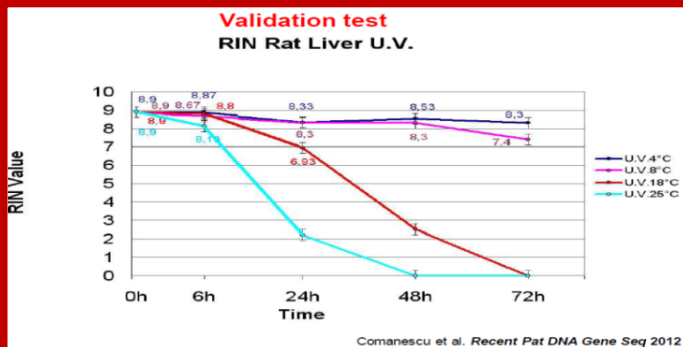
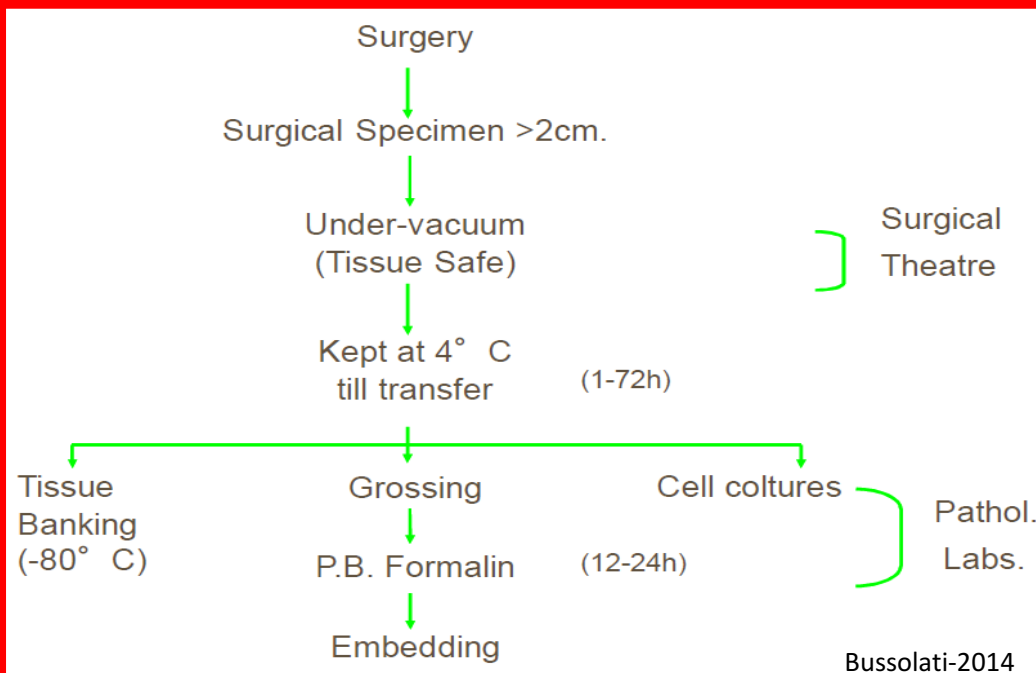
*\*Accidentally freezing and thawing the tissue (e.g., by using cool packs in a wrong manner) can lead to protein degradation*

# Tissues transport under vacuum

Structure, RNA, antigens preserved up to days



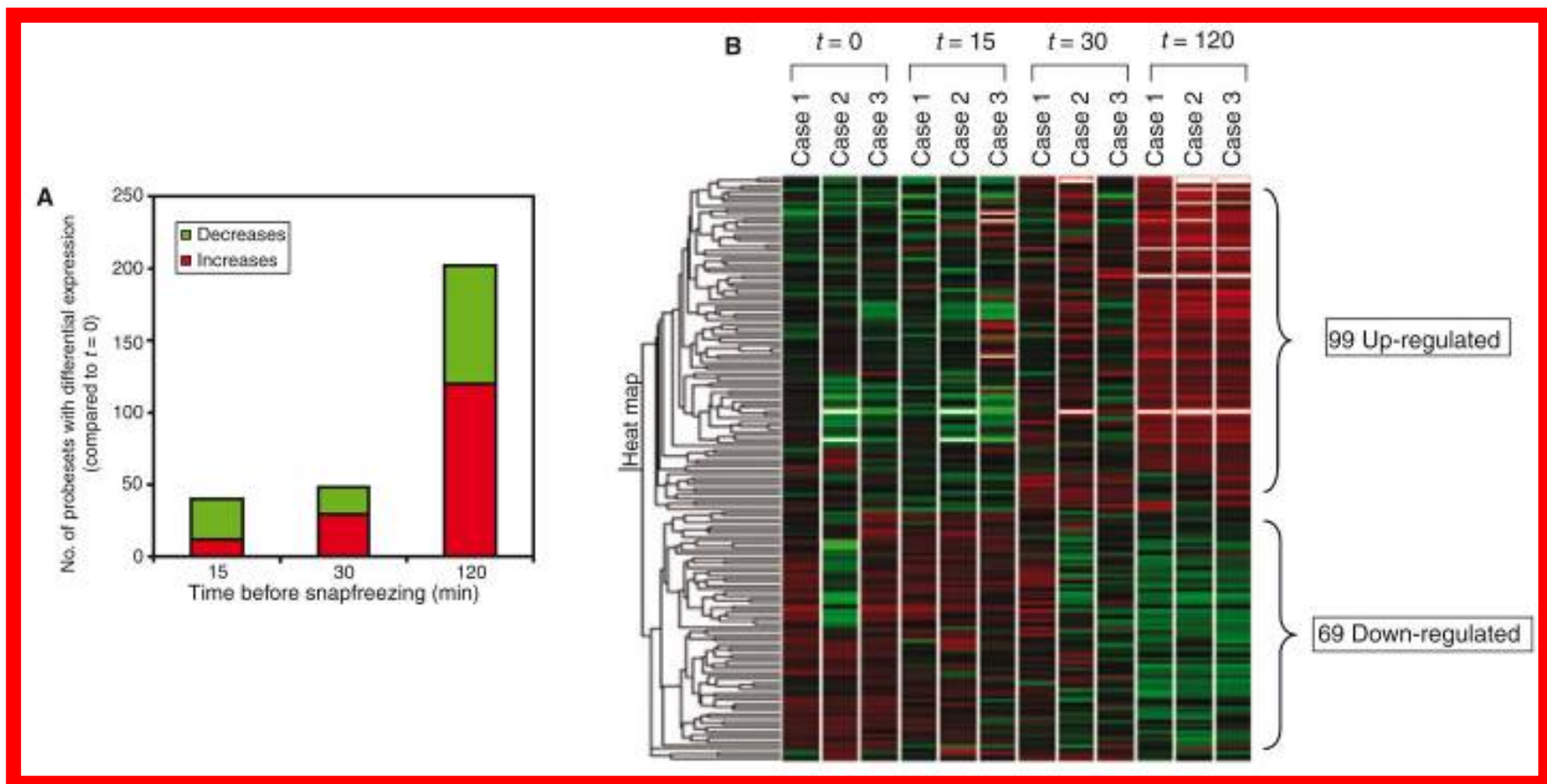
From B P Portier et al Modern Pathology 2012





*# Inducible genes: warm ischemia and cold ischemia can differently influence genes with increased or decreased expression, with changes in mRNA expression but also at the protein level.*

*# On the other hand, many genes can be totally indifferent to ischemia.*



Histopathology 2010, 56, 240–250. DOI: 10.1111/j.1365-2559.2009.03470.x

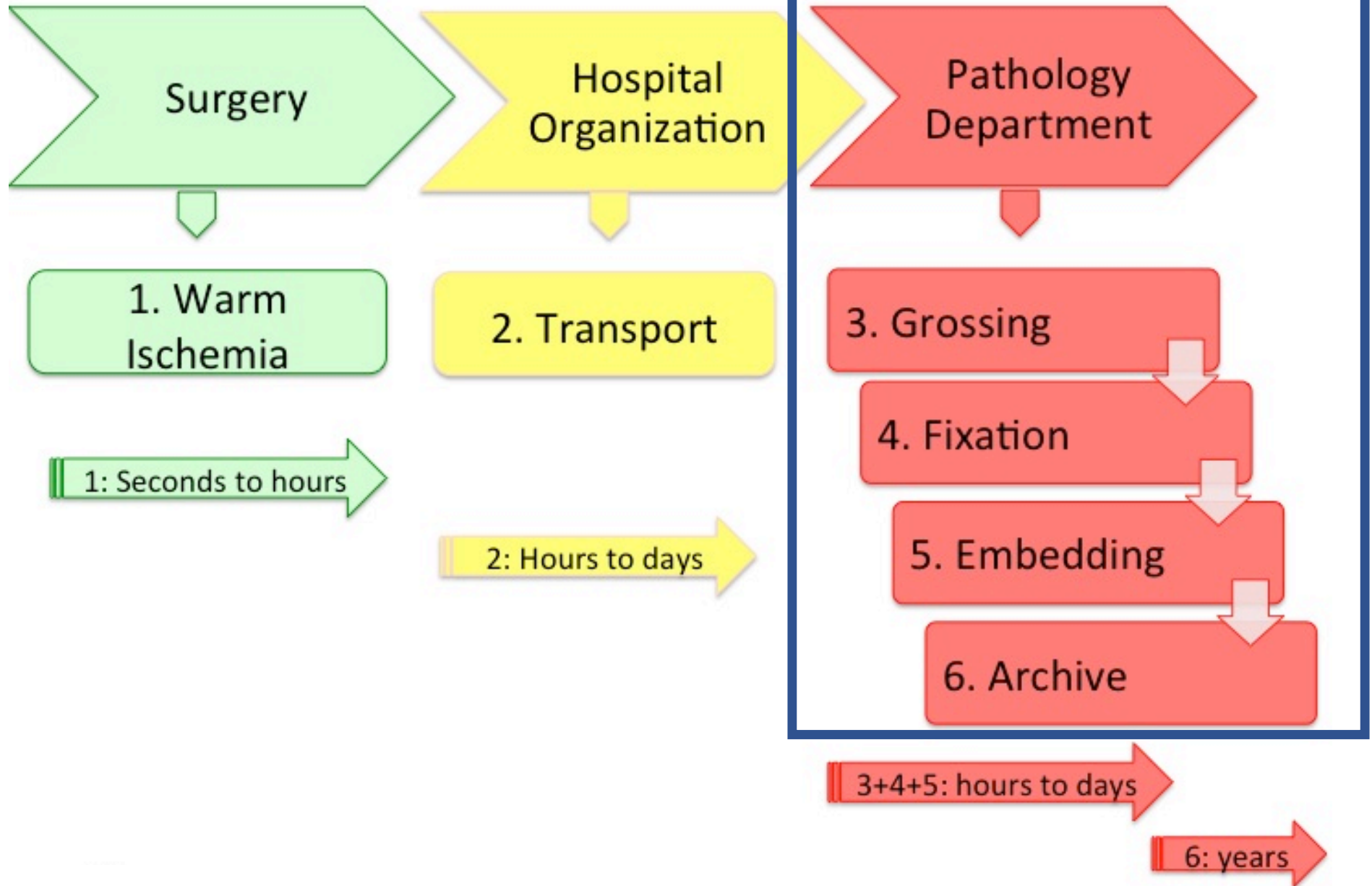
### Gene expression in colorectal neoplasia: modifications induced by tissue ischaemic time and tissue handling protocol

Susan E Bray, Fiona E M Paulin, Siew Chinn Fong, Lee Baker, Frank A Carey,<sup>1</sup>  
David A Levison,<sup>1</sup> Robert J C Steele & Neil M Kernohan<sup>1</sup>

# ***Preamanalytical Conditions***

**Outside the pathology lab**

**Inside the pathology lab**





# CLINICAL TISSUES – ARCHIVE TISSUES

**DIAGNOSTICS**

**SURGERY**

**HUMAN TISSUES  
DIAGNOSTIC FLOW**

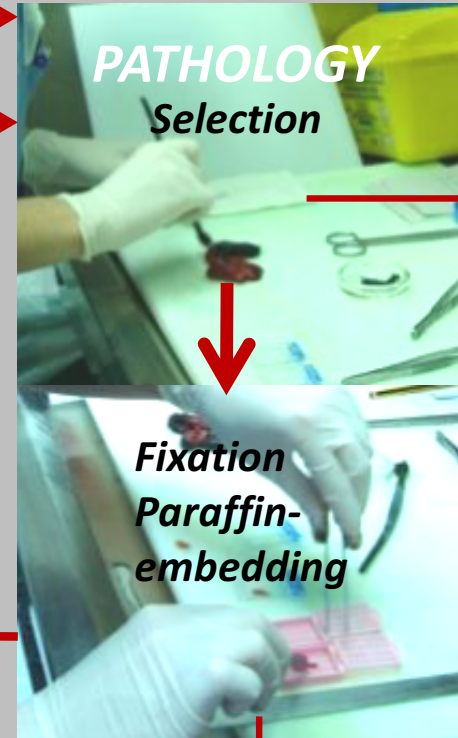
**PATHOLOGY**  
Selection

Fixation  
Paraffin-  
embedding

Slides  
preparation

**Surgical Left-over**

**PATHOLOGY  
ARCHIVES**



## ***Formalin penetration rate/equilibrium RNA and protein artifacts***

**#** The formalin penetration rate does not correspond to fixation, the formaldehyde-methylene glycol equilibrium shifts towards formaldehyde raise the effective concentration of the active molecule.  $\text{CH}_2=\text{O} + \text{H}_2\text{O} \rightleftharpoons \text{OH}-(\text{CH}_2\text{O})-\text{H}$

**#** Molecules are modified by fixation in formalin with artifacts: the formation of methylene bridges among different aminoacid residues, RNA hydrolysis and nucleic acids mechanical rupture is due to molecule stiffening from crosslinking.

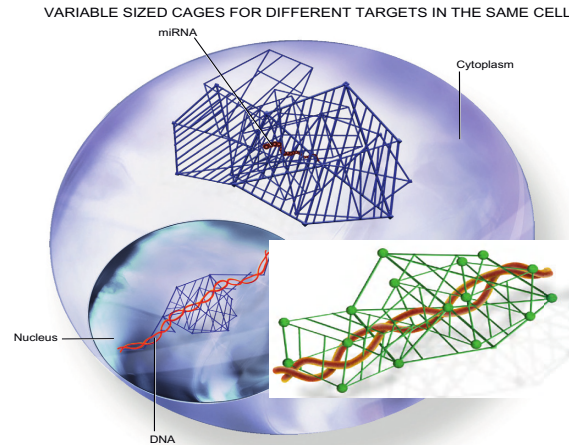
**#** Alterations are quantitatively related to time of fixation.

**#** Due to the thickness of tissues, alterations are not uniform: from over-fixation in the outer part, to hypoxia in the inner part of the tissue at the same time, alterations are complex.

# FORMALIN ARTIFACTS - 1

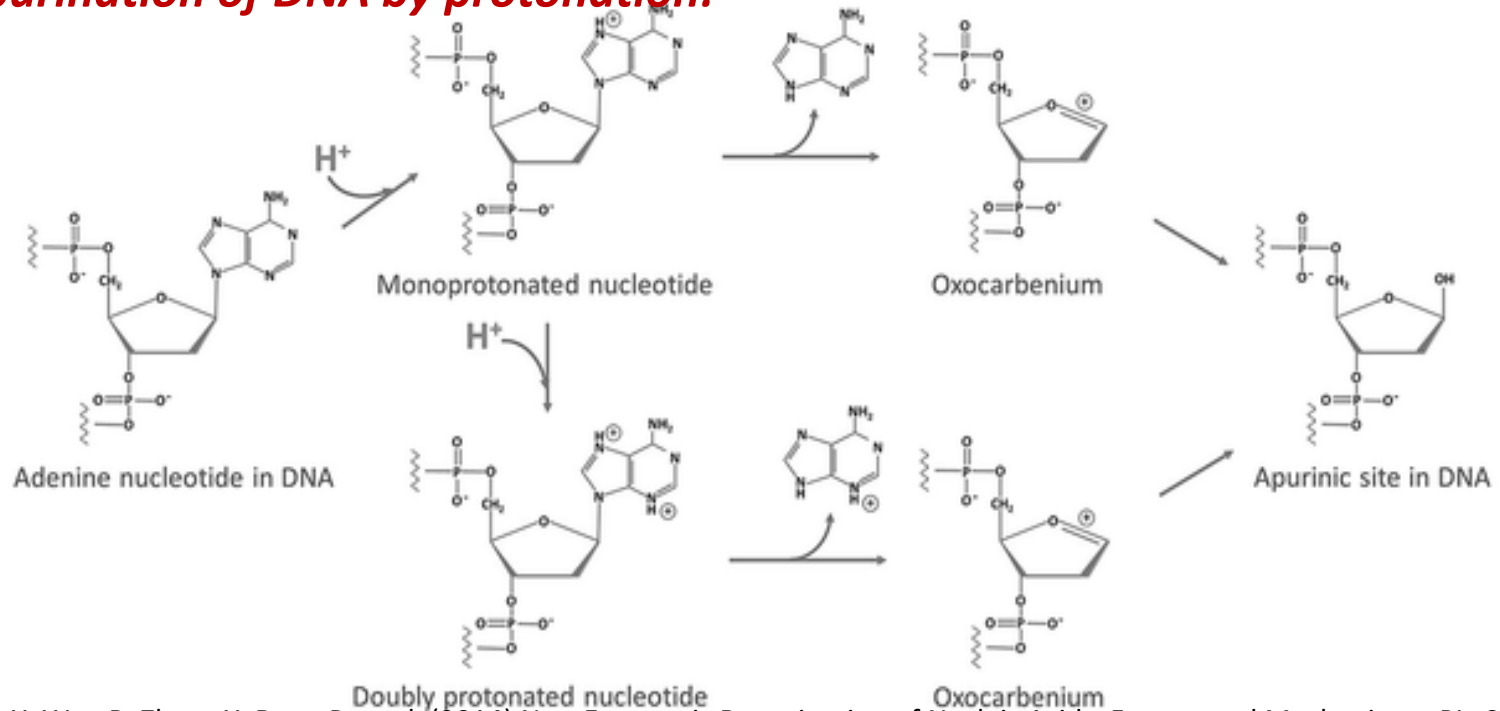
## DNA:

### 1. Mechanical rupture:



Gerard J. Nuovo, In Situ Molecular Pathology and Co-Expression Analyses 2013

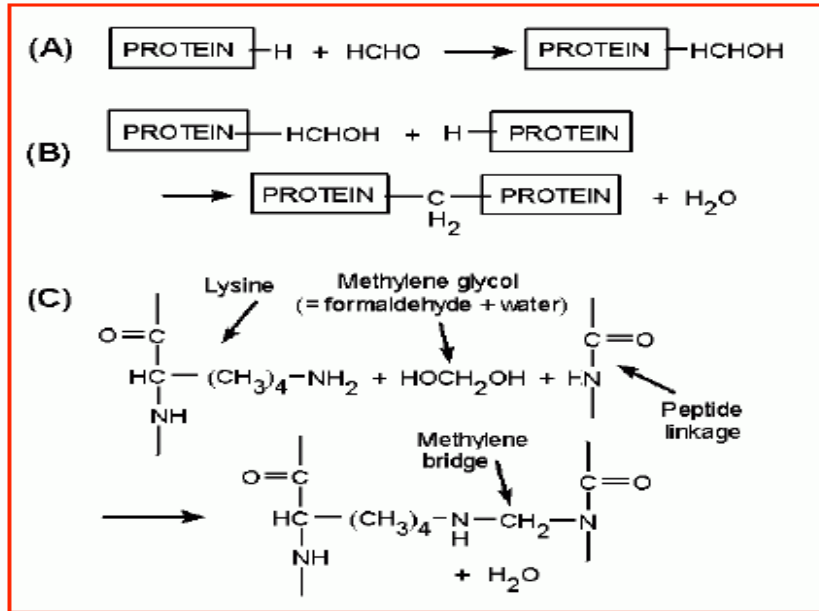
### 2. Depurination of DNA by protonation:



An R, Jia Y, Wan B, Zhang Y, Dong P, et al. (2014) Non-Enzymatic Depurination of Nucleic Acids: Factors and Mechanisms. PLoS ONE 9(12): e115950.

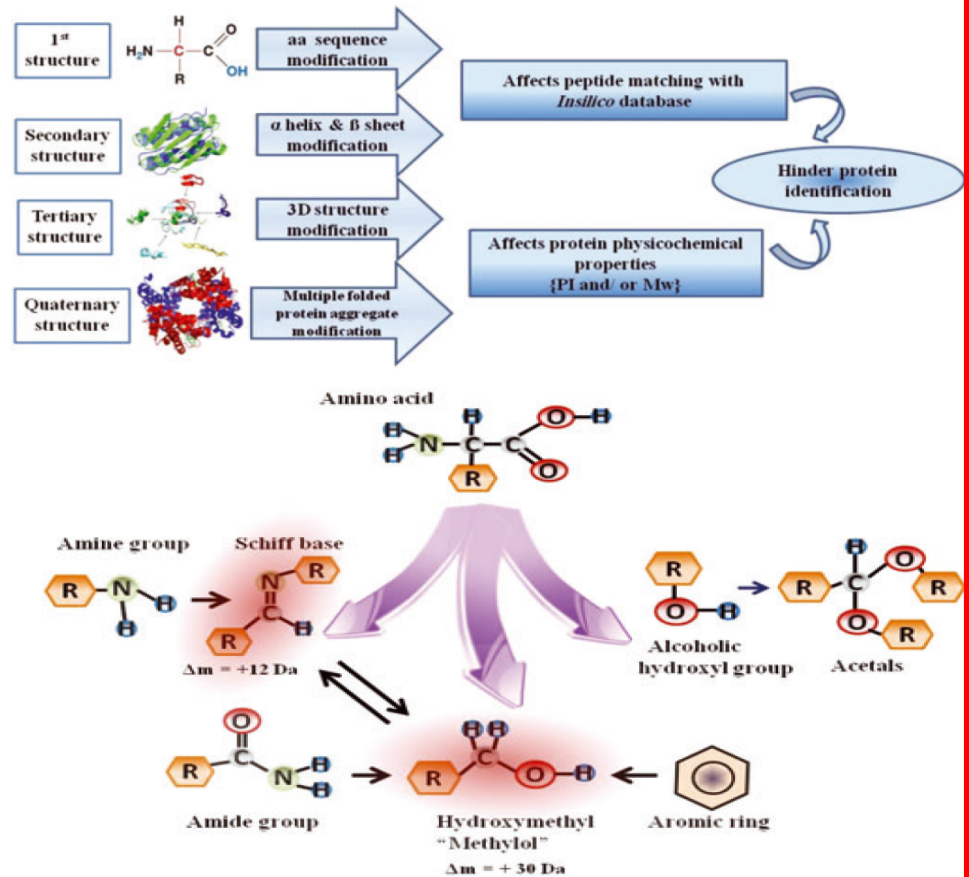
# FORMALIN ARTIFACTS - 2

## PROTEINS:



Kiernan, JA. Formaldehyde, formalin, paraformaldehyde and glutaraldehyde: What they are and what they do. *Microscopy Today* 00-1 pp. 8-12 (2000).

- Addition of formaldehyde molecules to proteins
- Methylene bridge formation between proteins
- Cross binding between lysine and methylene



## RNA:

Addition of methylol groups ( $\text{CH}_2\text{-OH}$ ) during formalin fixation to bases makes RNA resistant to RT. All the 4 bases show this type of alteration but to a different level (40% A ÷ 4% U). Reverse transcription efficiency is sequence related.

>>> RNA Demodification (20 min in TE buffer at 70°C)

E. Nardon, M. Donada, S. Bonin, I. Dotti, and G. Stanta. "Higher random oligos concentration improves reverse transcription yield of cDNA from biptic tissues and quantitative RT-PCR reliability". *Exp Mol Pathol* 87:146–151;2009

Sameh Magdeldin and Tadashi Yamamoto *Proteomics* 2012, 12, 1045–1058



# QUALITY AND QUANTITY OF DEGRADED RNA

## RNA

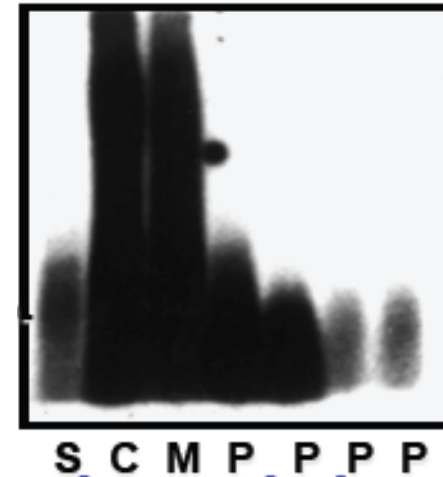
**#Formalin:** Biopsy < 120 – 200 bases  
Autopsy < 70 bases

**#Bouin solution** Biopsy < 70 bases

**#Alcoholic fix.:** Biopsy < about 600 bases

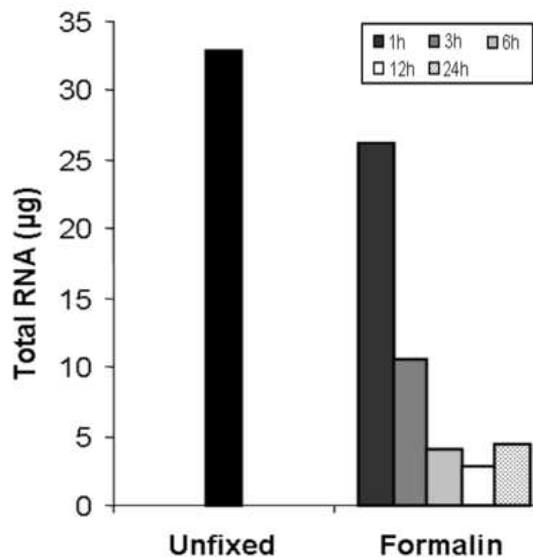
## DNA

**#Formalin:** Biopsy < 200-400 bases  
Autopsy < 150 bases



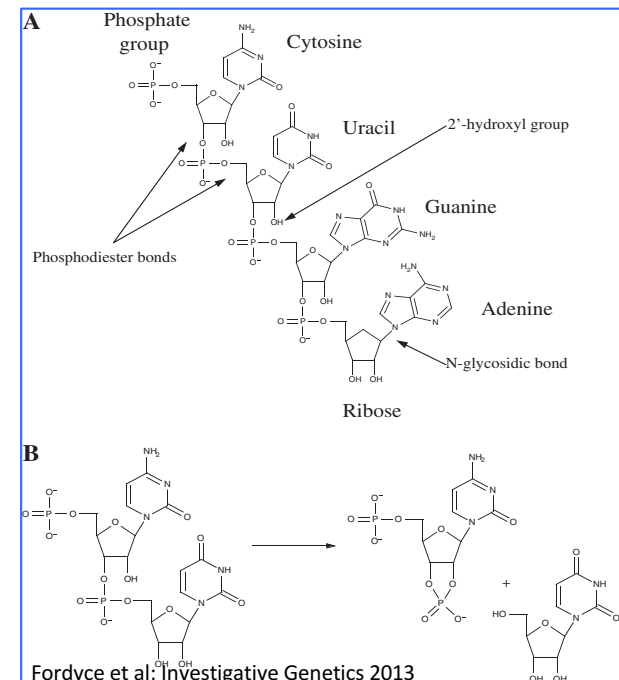
Bonin S., Petrera F., Stanta G. PCR and RT-PCR Analysis In Archival Postmortem Tissues. In Fuchs J, Podda M. Encyclopedia Of Diagnostic Genomics And Proteomics. M. Dekker, New York: 985-988; 2005 "

G. Stanta and C. Schneider, RNA extracted from paraffin-embedded human tissues is amenable to analysis by PCR amplification. Biotechniques, 11:304-308,1991."



## RNA degradation in FFPE is a cumulative effect:

- RNases activity in pre- and after fixation time
- hypoxia in the preanalysis time
- fixation with mechanical rupture due to molecule stiffening from crosslinking
- alkaline pH procedures
- high temperatures: RNA is thermodynamically less stable than DNA because the 2'-OH group on the ribose ring promotes the hydrolytic reaction



I.Dotti, S.Bonin, G. Basili, E. Nardon, A. Balani, S. Siracusano, F. Zanconati, S. Palmisano, N. De Manzini and G. Stanta. "Effects of formalin, methacarn and FineFIX fixatives on RNA preservation". Diagn Mol Pathol 19:112-122; 2010  
S Bonin, F Petrera, G Stanta, "PCR and RT-PCR Analysis in Archival Postmortem Tissues" in "Encyclopedia of Medical Genomics and Proteomics" Marcel Dekker, New York: 985-988; 2005

# Preanalytical Conditions

Outside the pathology lab

Inside the pathology lab



1. Warm

3. Grossing

4. Fixation

5. Embedding

6. Archive

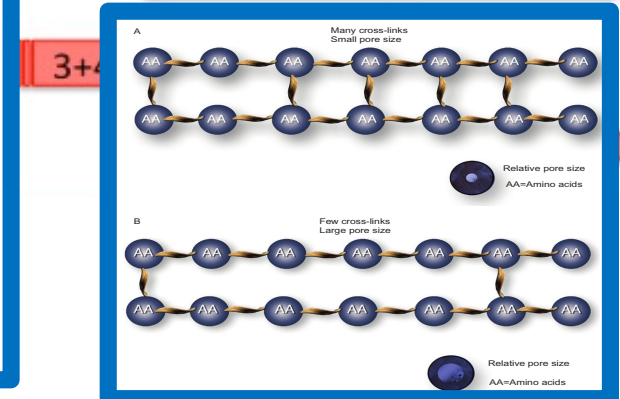
*#Standard buffered formalin solution with checked pH, because it can be oxidized to formic acid.*

*#Tissue specimens thickness should be to a maximum of 5 mm with fixation time between 12h and 24h (maximum 72 h, over week-end).*

*#For larger specimens slow penetration of formalin can result in a non-homogeneous fixation process.*

*#Fixation for more than 24 h can lead to increased crosslinking phenomena with lower molecular accessibility (Wolff, C et al, 2011, PloS One Journal, 6, e16353).*

*#The buffered formalin solution to tissue ratio should be from minimum of 4:1 to 10:1.*



# IS IT POSSIBLE TO IMPROVE FORMALIN FIXATION?

❖ Controlled fixation time ➡



❖ Cold fixation (longer fixation at low T)



*# Protein and RNA degradation would be inhibited by maintaining low temperature throughout the fixation process.*

Bussolati G et al. PLOS ONE 2011  
Chafin D et al PLOS ONE 2013

# Preanalytical Conditions

## Outside the pathology lab



Hospital  
Organization

## Inside the pathology lab

Pathology  
Department

# The inner poorly fixed hypoxic areas can also be affected by inclusion procedures:

- alcohol treatment coagulates the proteins
- inclusion temperatures can affect more sensitive antigens and RNA

# During processing, the tissue sample is dehydrated and water is replaced with paraffin wax. It is essential to replace water completely, since residual water leads to tissue degradation during storage.

3. Grossing

4. Fixation

5. Embedding

6. Archive

3+4+5: hours to days

6: years



# Preanalytical Conditions

## Outside the pathology lab

## Inside the pathology lab



*#Storage time may influence the retrieval of antigens and quality of RNA (Balgley, B. (2009) Journal of Proteome Research, 8, 917–925)*

*#While histology is not affected by storage, protein and RNA degradation may increase with increasing storage time, especially for long time*

*(Wolff, C.. (2011) PloS One Journal, 6, e16353.)*

*#Storage conditions, such as humidity and temperature, can have an impact on protein and RNA amounts and quality*

*(Thompson, S (2013). Proteomics - Clinical Applications, 7, 241–51)*

3. Grossing

4. Fixation

5. Embedding

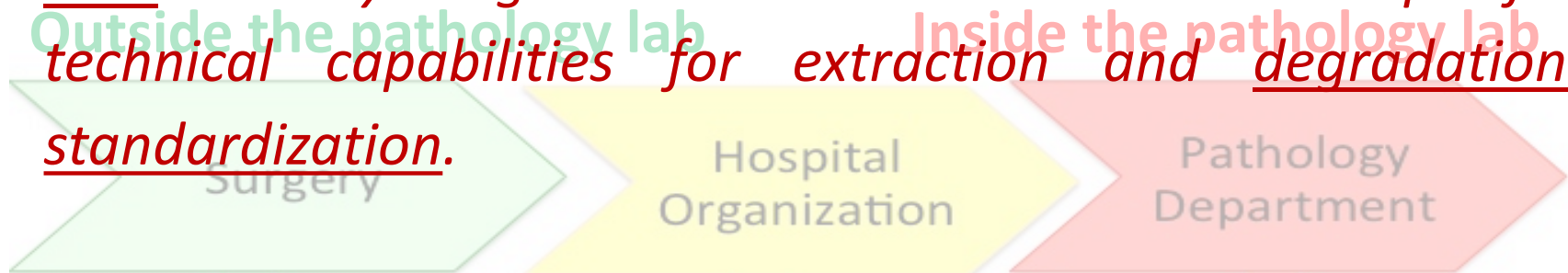
6. Archive

3+4+5: hours to days

6: years

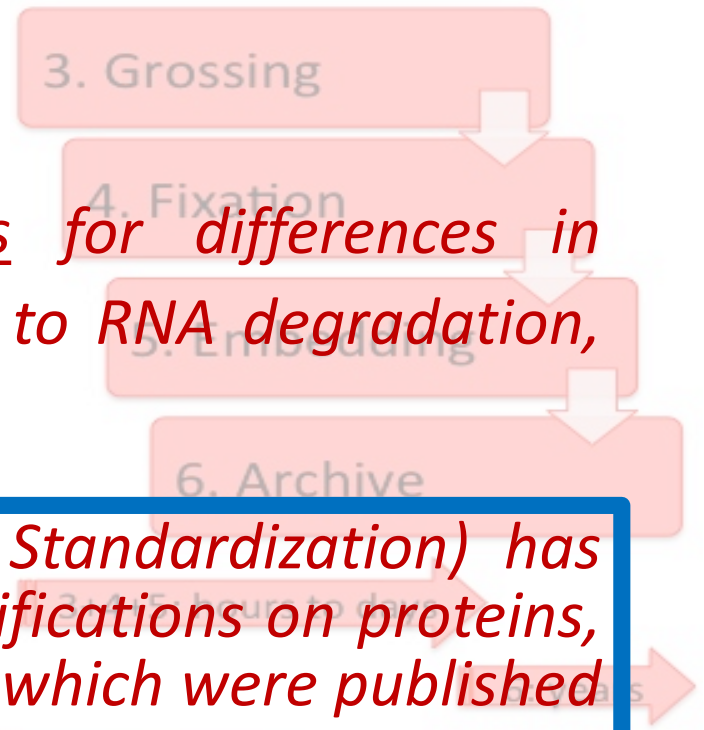
# Preanalytical Conditions

RNA is always degraded in FFPE tissues and needs specific technical capabilities for extraction and degradation standardization.



There are genes whose expression is induced by warm and cold ischemia and others that are not.

There are also technical artefacts for differences in detected expression also not related to RNA degradation, like different sequences for RT.



CEN (the European Committee for Standardization) has developed preanalytic technical specifications on proteins, DNA and RNA in tissues to ISO 15189 which were published in 2015.

# Pre-analytical Conditions in IHC

Outside the pathology lab

Inside the pathology lab

**Proteins** during the preanalytical phase may be categorized into three groups:

- (1) predictable stable;
- (2) predictable unstable;
- (3) unpredictable.

All these possible alterations, together with methods and interpretation pitfalls, make specific external and internal quality assessment and audit procedures mandatory for IHC and any protein analysis.

CEN (the European Committee for Standardization) has developed preanalytic technical specifications on proteins, DNA and RNA in tissues to ISO 15189 which were published in 2015.

# *Clinical research irreproducibility*

```
graph TD; A[Clinical research irreproducibility] --> B[Technological complexity]; A --> C[Biological complexity]; B --> D["#Tissue and macromolecule pre-analytical preservation<br/>#Selection and standardization of analytical procedures"]; C --> E["#Heterogeneity at the clinical, morphological or molecular level"]; E --- F[BIOLOGICAL COMPLEXITY];
```

## ***SOURCES OF VARIABILITY***

*#Tissue and macromolecule  
pre-analytical preservation*

*#Selection and  
standardization of analytical  
procedures*

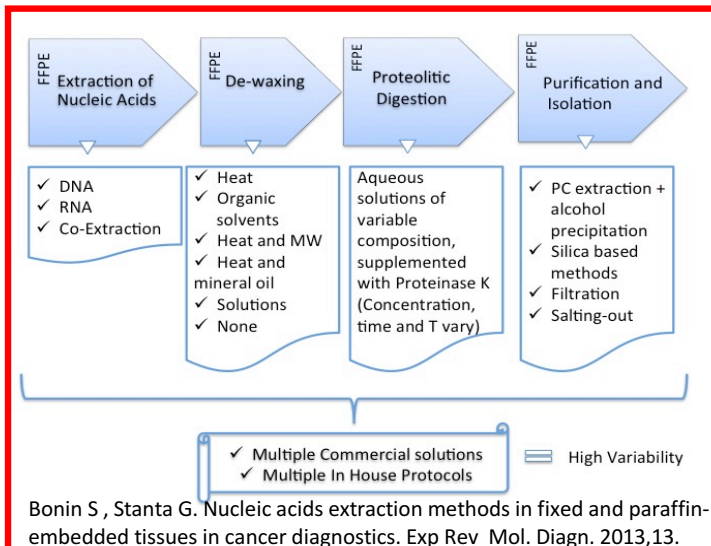
***Technological  
complexity***

***Biological  
complexity***

*#Heterogeneity at the  
clinical, morphological or  
molecular level*

***BIOLOGICAL COMPLEXITY***





## SOURCES OF VARIABILITY

### #Selection and standardization of analytical procedures



### A recipe for disaster

Manufacturers of commercial reagents should follow scientific norms and be open about the ingredients of their products, says Anna Git.

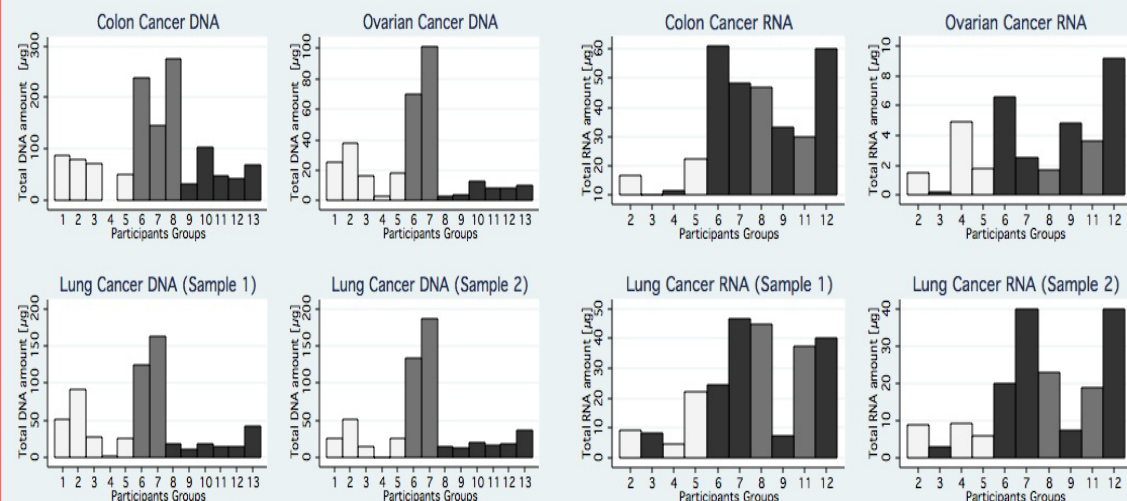
Earlier this year, my colleagues and I experienced every scientist's worst nightmare. Twelve months of experiments were deemed useless after we showed that a recommended negative control for thermally synthesized stretches of RNA (microRNA mimics), bought from a biotechnology company, was inappropriate. The sequence was too short, leading to results that were impossible to interpret, if not just wrong. Because the company didn't reveal much information about the product, we only discovered the discrepancy intuitively after testing many microRNAs of known sequence, and observing a length-dependent activity among them.

This is the worst in a long line of incidents that we have experienced as a result of the sweeping confidentiality imposed by manufacturers of laboratory reagents, who, for the most part, do not provide full details about the contents of their chemicals, enzymes or kits. This lack of transparency forces researchers to waste time chasing information, restricts the types of experiments they can and cannot do, and most troublingly, causes them unknowingly to perform inappropriate experiments and publish misleading results.

To try to decipher the ingredients of commercial products, my colleagues and I have tested pH and conductivity, signed confidentiality agreements to receive extra information not on the label and discarded experiments in which unknown ingredients impeded subsequent reactions. We are on first-name terms with many sympathetic scientists who work in research and development (R&D) for commercial vendors, and who occasionally whisper crucial details off the record.

This secrecy stands in stark contrast to the current practice of scientific publications. No self-respecting referee or journal would accept a research paper in which the authors relied on processes, substances or sequences that they had created themselves but did not describe in detail. Yet this is acceptable.

20 APRIL 2013 | 101-104 | NATURE | 103



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

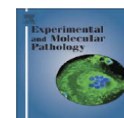
Experimental and Molecular Pathology 87 (2009) 146–151



Contents lists available at ScienceDirect

Experimental and Molecular Pathology

journal homepage: [www.elsevier.com/locate/yexmp](http://www.elsevier.com/locate/yexmp)



Higher random oligo concentration improves reverse transcription yield of cDNA from biopsic tissues and quantitative RT-PCR reliability

Ermanno Nardon <sup>a,b</sup>, Marisa Donada <sup>b</sup>, Serena Bonin <sup>a,b</sup>, Isabella Dotti <sup>a</sup>, Giorgio Stanta <sup>a,b,\*</sup>

<sup>a</sup> Department of Clinical, Morphological and Technological Sciences, University of Trieste, Italy

<sup>b</sup> International Centre for Genetic Engineering and Biotechnology, Padriciano, Trieste, Italy

**“Reverse transcription yield, indeed, can vary up to 100-fold depending on priming strategy, on the used enzyme, on the starting quantity of target RNA and even on the type of sequence that is going to be detected.”**

**Table 4**  
Summary of case study results.

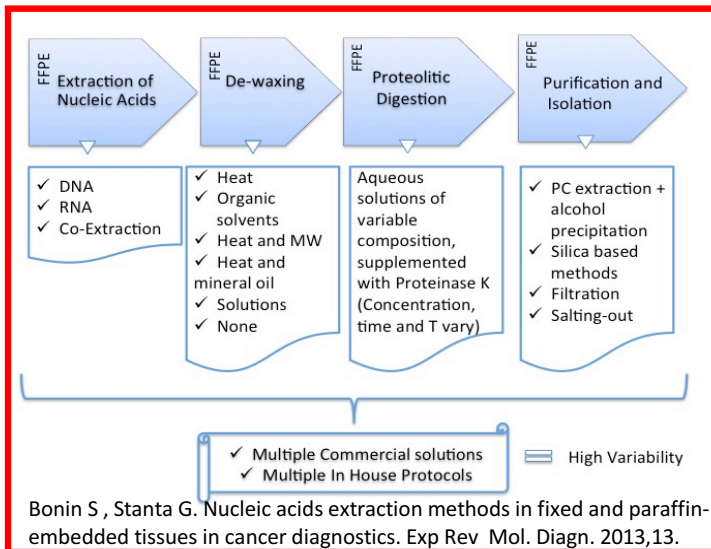
|                                               | ACTB gene           |      |             | TS gene             |      |             |
|-----------------------------------------------|---------------------|------|-------------|---------------------|------|-------------|
|                                               | Mean C <sub>t</sub> | SD   | Min.–Max.   | Mean C <sub>t</sub> | SD   | Min.–Max.   |
| 0.14 nmol random hexamers RT                  | 23.45               | 0.79 | 22.58–24.94 | 34.34               | 0.65 | 33.30–35.26 |
| 3.35 nmol random hexamers RT                  | 21.75               | 0.78 | 20.60–23.43 | 32.16               | 0.73 | 30.69–33.24 |
| Difference between matched pairs <sup>a</sup> | 1.69                | 0.26 | 1.33–2.08   | 2.18                | 0.28 | 1.77–2.71   |

Case study samples were reverse transcribed in two different conditions and TS and ACTB genes were qRT-PCR amplified. Mean C<sub>t</sub> is the mean C<sub>t</sub> value of the 12 samples and SD its standard deviation. Min.–Max. is the range of the observed C<sub>t</sub>.

<sup>a</sup> Value obtained subtracting the C<sub>t</sub> of a sample submitted to RT with the lower primer concentration from the C<sub>t</sub> of the same sample submitted to RT with the higher primer concentration.

G. Stanta





## SOURCES OF VARIABILITY

### #Selection and standardization of analytical procedures



Stant information on the myriad kits and reagents purchased from various manufacturers.

**A recipe**

Manufacturers of commercial products should be open about the ingredients of their products, says Anna Gill.

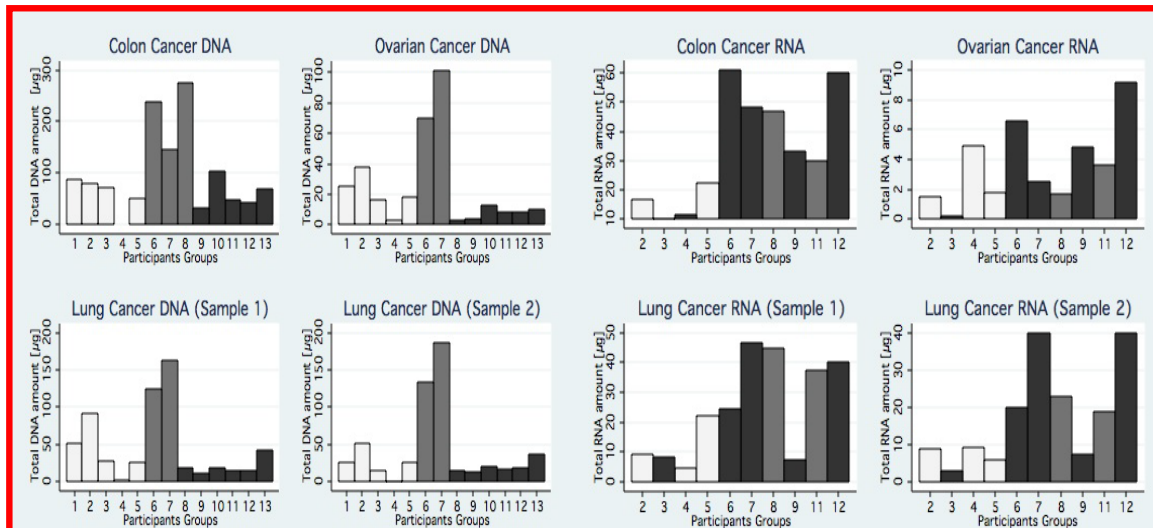
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Serena Bonin, Falk Hlubek, Jean Benhattar, Carsten Denkert, Manfred Dietel, Pedro L. Fernandez, Gerald Höfler, Hannelore Kothmaier, Bozo Kruslin, Chiara Maria Mazzanti, Aurel Perren, Helmuth Popper, Aldo Scarpa, Paula Soares, Giorgio Stanta and Patricia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



# SOPs-IQCs!!!

cDNA

priming  
even on

Table 4  
Summary of case study results.

|                                               | ACTB gene           |      |             | TS gene             |      |             |
|-----------------------------------------------|---------------------|------|-------------|---------------------|------|-------------|
|                                               | Mean C <sub>t</sub> | SD   | Min.-Max.   | Mean C <sub>t</sub> | SD   | Min.-Max.   |
| 0.14 nmol random hexamers RT                  | 23.45               | 0.79 | 22.58-24.94 | 34.34               | 0.65 | 33.30-35.26 |
| 3.35 nmol random hexamers RT                  | 21.75               | 0.78 | 20.60-23.43 | 32.16               | 0.73 | 30.69-33.24 |
| Difference between matched pairs <sup>a</sup> | 1.69                | 0.26 | 1.33-2.08   | 2.18                | 0.28 | 1.77-2.71   |

Case study samples were reverse transcribed in two different conditions and TS and ACTB genes were qRT-PCR amplified. Mean C<sub>t</sub> is the mean C<sub>t</sub> value of the 12 samples and SD is standard deviation. Min.-Max. is the range of the observed C<sub>t</sub>s.

<sup>a</sup> Value obtained subtracting the C<sub>t</sub> of a sample submitted to RT with the lower primer concentration from the C<sub>t</sub> of the same sample submitted to RT with the higher primer concentration.

# Why pre-analytics? Why in FFPE?



**F**ormalin 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<sup>1</sup> 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 under-addressed in the scientific literature. While technological

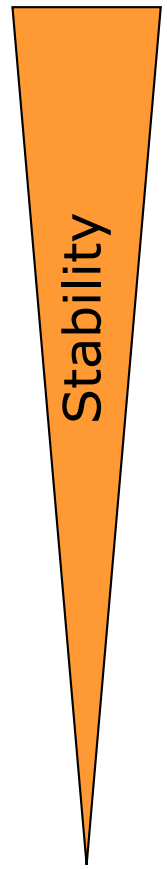
Arch Pathol Lab Med—Vol 138, November 2014

## 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



Surgery

Hospital  
Organization

Pathology  
Department

1. Warm  
Ischemia

2. Transport

3. Grossing

4. Fixation

5. Embedding

6. Archive

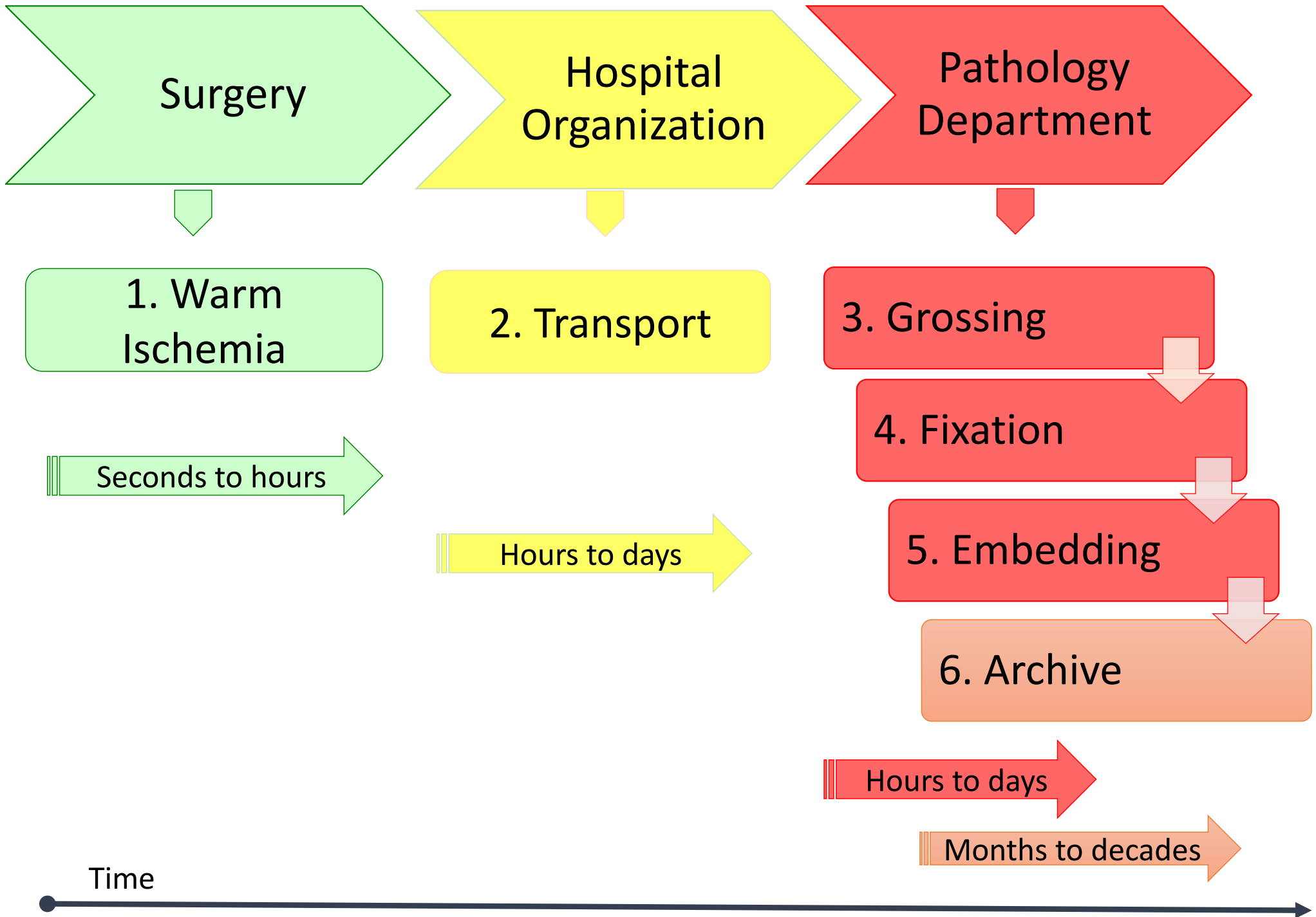
Seconds to hours

Hours to days

Hours to days

Months to decades

Time



# SPIDIA→ 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 for FFPE tissues

TECHNICAL SPECIFICATION  
SPÉCIFICATION TECHNIQUE  
TECHNISCHE SPEZIFIKATION

**CEN/TS 16827-1**

August 2015

ICS 11.100.10

English Version

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

Tests de diagnostic moléculaire in vitro - Spécifications relatives aux processus préanalytiques pour les tissus FFPE - Partie 1: ARN extrait

Molekularanalytische in-vitro-diagnostische Verfahren - Spezifikationen für präanalytische Prozesse für FFPE-Gewebeproben - Teil 1: Isolierte RNS

This Technical Specification (CEN/TS) was approved by CEN on 6 July 2015 for provisional application.

The period of validity of this CEN/TS is limited initially to three years. After two years the members of CEN will be requested to submit their comments, particularly on the question whether the CEN/TS can be converted into a European Standard.

CEN members are required to announce the existence of this CEN/TS in the same way as for an EN and to make the CEN/TS available promptly at national level in an appropriate form. It is permissible to keep conflicting national standards in force (in parallel to the CEN/TS) until the final decision about the possible conversion of the CEN/TS into an EN is reached.

CEN members are the national standards bodies of Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, Former Yugoslav Republic of Macedonia, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and United Kingdom.



EUROPEAN COMMITTEE FOR STANDARDIZATION  
COMITÉ EUROPÉEN DE NORMALISATION  
EUROPÄISCHES KOMITEE FÜR NORMUNG

TECHNICAL SPECIFICATION  
SPÉCIFICATION TECHNIQUE  
TECHNISCHE SPEZIFIKATION

**CEN/TS 16827-2**

August 2015

ICS 11.100.10

English Version

**Molecular in vitro diagnostic examinations - Specifications for pre-examination processes for FFPE tissue - Part 2: Isolated proteins**

Tests de diagnostic moléculaire in vitro - Spécifications pour les processus préanalytiques pour tissu FFPE - Partie 2: Protéines extraites

Molekularanalytische in-vitro-diagnostische Verfahren - Spezifikationen für präanalytische Prozesse für FFPE-Gewebeproben - Teil 2: Isolierte Proteine

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EUROPEAN COMMITTEE FOR STANDARDIZATION  
COMITÉ EUROPÉEN DE NORMALISATION  
EUROPÄISCHES KOMITEE FÜR NORMUNG

CEN-CENELEC Management Centre: Avenue Marnix 17, B-1000 Brussels

TECHNICAL SPECIFICATION  
SPÉCIFICATION TECHNIQUE  
TECHNISCHE SPEZIFIKATION

**CEN/TS 16827-3**

August 2015

ICS 11.100.10

English Version

**Molecular in vitro diagnostic examinations - Specifications for pre-examination processes for FFPE tissue - Part 3: Isolated DNA**

Tests de diagnostic moléculaire in vitro - Spécifications relatives aux processus préanalytiques pour les tissus FFPE - Partie 3: ADN isolé

Molekularanalytische in-vitro-diagnostische Verfahren - Spezifikationen für präanalytische Prozesse für FFPE-Gewebeproben - Teil 3: Isolierte DNS

This Technical Specification (CEN/TS) was approved by CEN on 6 July 2015 for provisional application.

The period of validity of this CEN/TS is limited initially to three years. After two years the members of CEN will be requested to submit their comments, particularly on the question whether the CEN/TS can be converted into a European Standard.

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EUROPEAN COMMITTEE FOR STANDARDIZATION  
COMITÉ EUROPÉEN DE NORMALISATION  
EUROPÄISCHES KOMITEE FÜR NORMUNG

# CEN/TS- European Technical Specification for FFPE tissues

| Contents                                                                                                                                                       | Page |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------|------|
| European foreword .....                                                                                                                                        | 3    |
| Introduction .....                                                                                                                                             | 4    |
| 1 Scope .....                                                                                                                                                  | 5    |
| 2 Normative references .....                                                                                                                                   | 5    |
| 3 Terms and definitions .....                                                                                                                                  | 5    |
| 4 General considerations .....                                                                                                                                 | 7    |
| 5 Outside the laboratory .....                                                                                                                                 | 8    |
| 5.1 Primary tissue collection manual .....                                                                                                                     | 8    |
| 5.1.1 Information about the primary sample donor .....                                                                                                         | 8    |
| 5.1.2 Information on the primary tissue sample .....                                                                                                           | 8    |
| 5.1.3 Information on the primary tissue sample processing .....                                                                                                | 8    |
| 5.2 Transport requirements .....                                                                                                                               | 9    |
| 6 Inside the laboratory .....                                                                                                                                  | 9    |
| 6.1 Information on the primary tissue sample receipt .....                                                                                                     | 9    |
| 6.2 Formalin fixation of the specimen .....                                                                                                                    | 9    |
| 6.3 Evaluation of the pathology of the specimen and selection of the sample .....                                                                              | 10   |
| 6.4 Post-fixation of frozen samples .....                                                                                                                      | 11   |
| 6.5 Processing and paraffin embedding .....                                                                                                                    | 11   |
| 6.6 Storage requirements .....                                                                                                                                 | 12   |
| 6.7 Isolation of the total RNA .....                                                                                                                           | 12   |
| 6.7.1 General .....                                                                                                                                            | 12   |
| 6.7.2 General information for RNA isolation procedures .....                                                                                                   | 12   |
| 6.7.3 Using commercial kits .....                                                                                                                              | 13   |
| 6.7.4 Using the laboratories' own protocols .....                                                                                                              | 13   |
| 6.8 Quantity and quality assessment of isolated RNA .....                                                                                                      | 14   |
| 6.9 Storage of isolated RNA .....                                                                                                                              | 14   |
| Annex A (informative) Quality control of RNA extracted from formalin fixed and paraffin embedded tissue samples: implications for RT-qPCR based analyses ..... | 15   |
| A.1 Summary .....                                                                                                                                              | 15   |
| A.2 Results .....                                                                                                                                              | 15   |
| A.2.1 Time dependency of RNA integrity .....                                                                                                                   | 15   |
| A.2.2 Impact of formalin-fixation on cDNA synthesis efficiency .....                                                                                           | 16   |
| A.2.3 Fixation and storage introduces major gene-to-gene variations in RT-qPCR .....                                                                           | 17   |
| A.2.4 Impact of storage conditions of FFPE blocks on RNA integrity .....                                                                                       | 18   |
| A.3 Conclusions .....                                                                                                                                          | 18   |
| A.4 Further reading .....                                                                                                                                      | 19   |
| Bibliography .....                                                                                                                                             | 20   |

# 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

## Target groups

- ✓ *In-vitro* diagnostic laboratories
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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!](#)

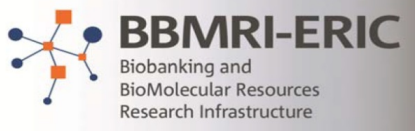
Self Assessment Surveys

[Access](#)



# BBMRI-ERIC Work Programme 2016

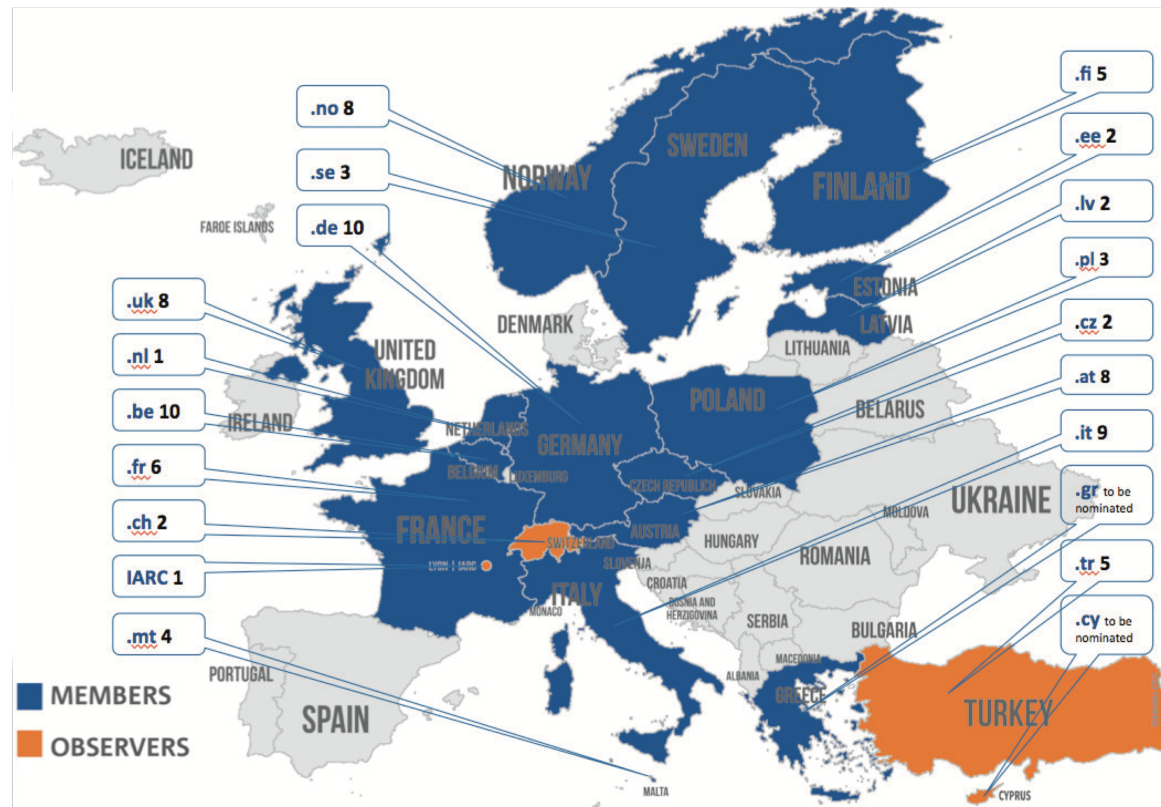
## Quality Work Stream 2.1 CEN/TC 140 / ISO 212 Quality of the sample



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



European Committee  
for Standardization





# BBMRI-ERIC Self assessment Survey

Registration



Compliance Assessment



Model 1:  
Biobank internal  
use

## Self-Assessment Survey

**\*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>                    | <div><input type="radio"/> Yes</div> <div><input type="radio"/> No</div> <div>e.g. code</div> <div>reset</div>                                                         |
| 13)                                            | Health status of donor/patient documented should       | <div><input type="radio"/> Yes</div> <div><input type="radio"/> No</div> <div>e.g. healthy, disease type, concomitant disease</div> <div>reset</div>                   |
| 14)                                            | Medical treatment documented should                    | <div><input type="radio"/> Yes</div> <div><input type="radio"/> No</div> <div>e.g. anaesthetics, medications, surgical or diagnostic procedures</div> <div>reset</div> |
| Start of warm ischemia documented:<br>*****    |                                                        |                                                                                                                                                                        |
| 15)                                            | Date of vessel ligation/arterial clamping time should  | <div><input type="radio"/> Yes</div> <div><input type="radio"/> No</div> <div>reset</div>                                                                              |
| 16)                                            | Time of vessel ligation/arterial clamping time should> | <div><input type="radio"/> Yes</div> <div><input type="radio"/> No</div> <div>reset</div>                                                                              |
| Information on the primary tissue sample       |                                                        |                                                                                                                                                                        |
| Start of cold ischemia documented:<br>*****    |                                                        |                                                                                                                                                                        |
| 17)                                            | Date of tissue removal from the body shall             | <div><input type="radio"/> Yes</div> <div><input type="radio"/> No</div> <div>reset</div>                                                                              |
| 18)                                            | Time of tissue removal from the body shall             | <div><input type="radio"/> Yes</div> <div><input type="radio"/> No</div> <div>reset</div>                                                                              |
| Tissue type and condition documented:<br>***** |                                                        |                                                                                                                                                                        |
| 19)                                            | General tissue type and condition shall                | <div><input type="radio"/> Yes</div> <div><input type="radio"/> No</div> <div>reset</div>                                                                              |

|                                                                                     |                                                                                                         |                                                                                                                                                                                                           |
|-------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 20)                                                                                 | Organ of origin and location within shall                                                               | <div><input type="radio"/> Yes</div> <div><input type="radio"/> No</div> <div>reset</div>                                                                                                                 |
| Type and start of fixation documented:<br>*****<br>(if started outside the biobank) |                                                                                                         |                                                                                                                                                                                                           |
| 21)                                                                                 | Date of start shall                                                                                     | <div><input type="radio"/> Yes</div> <div><input type="radio"/> No</div> <div>reset</div>                                                                                                                 |
| 22)                                                                                 | Time of start shall                                                                                     | <div><input type="radio"/> Yes</div> <div><input type="radio"/> No</div> <div>reset</div>                                                                                                                 |
| 23)                                                                                 | Fixative type shall                                                                                     | <div><input type="radio"/> Yes</div> <div><input type="radio"/> No</div> <div>reset</div>                                                                                                                 |
| 24)                                                                                 | Fixative condition shall                                                                                | <div><input type="radio"/> Yes</div> <div><input type="radio"/> No</div> <div>reset</div>                                                                                                                 |
| Information on the primary tissue sample processing                                 |                                                                                                         |                                                                                                                                                                                                           |
| 25)                                                                                 | Modifications after removal from body documented shall                                                  | <div><input type="radio"/> Yes</div> <div><input type="radio"/> No</div> <div>e.g. labelling for specimen orientation such as ink-marking, stitches, incisions</div> <div>reset</div>                     |
| 26)                                                                                 | Selection/use of transport containers performed shall                                                   | <div><input type="radio"/> Yes</div> <div><input type="radio"/> No</div> <div>e.g. cooling box, vacuum packing</div> <div>reset</div>                                                                     |
| 27)                                                                                 | Selection/use of stabilisation procedures for transport of unfixed primary tissue performed shall       | <div><input type="radio"/> Yes</div> <div><input type="radio"/> No</div> <div>e.g. cooling methods, fixation</div> <div>reset</div>                                                                       |
| 28)                                                                                 | Labelling of the transport container performed shall                                                    | <div><input type="radio"/> Yes</div> <div><input type="radio"/> No</div> <div>e.g. registration number, barcode (1D ord 2D), primary sample type, quantity, organ origin of tissue</div> <div>reset</div> |
| 29)                                                                                 | Documented, when several aliquots of a single sample with different features are in one container shall | <div><input type="radio"/> Yes</div> <div><input type="radio"/> No</div> <div>reset</div>                                                                                                                 |

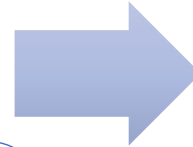
# **SPIDIA for personalised medicine: Standardisation and improvement of generic pre-analytical tools and procedures for in-vitro diagnostics**



- ✓ 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](http://www.spidia.eu)

CEN

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

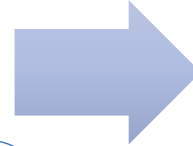


ISO/TC 212

- 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)

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)

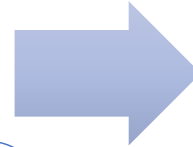




SPIDIA4P

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