UNIVERSITÀ
DEGLI STUDI DI TRIESTE

PREANALYTICAL CONDITIONS OF TISSUES

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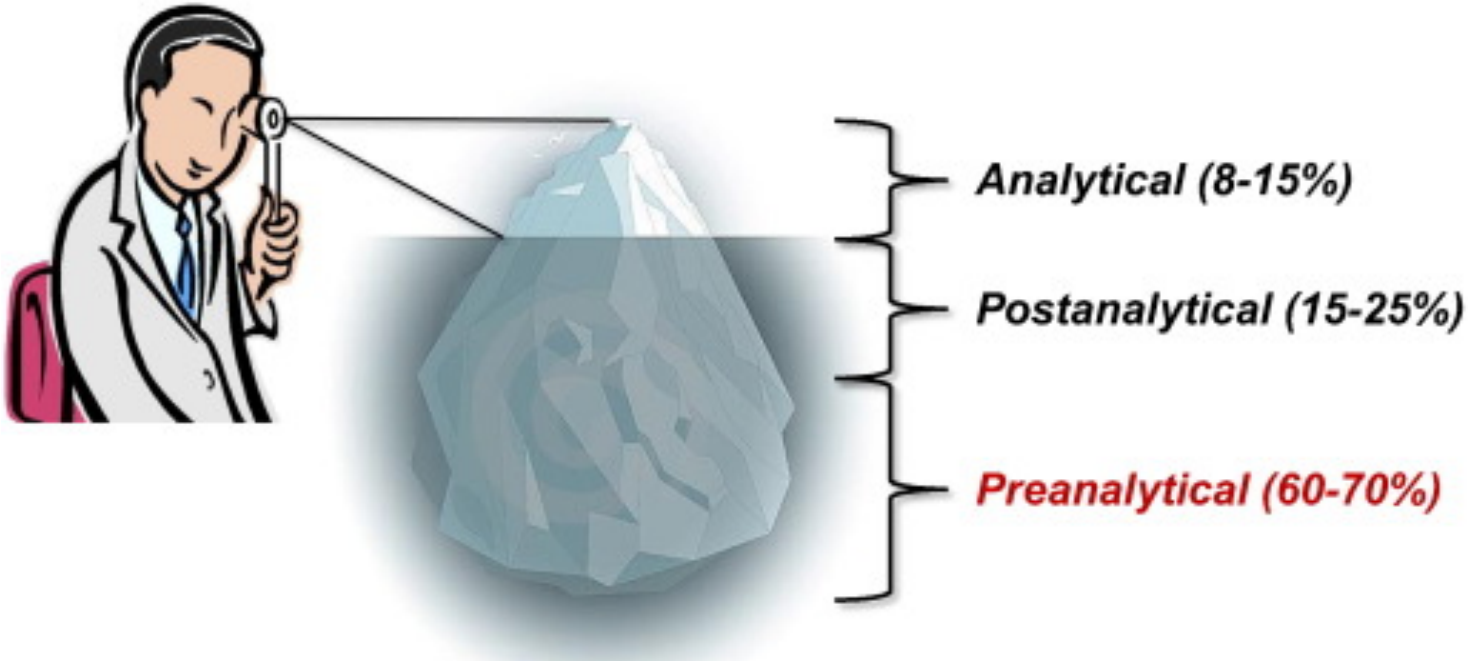
Why pre-analytics?

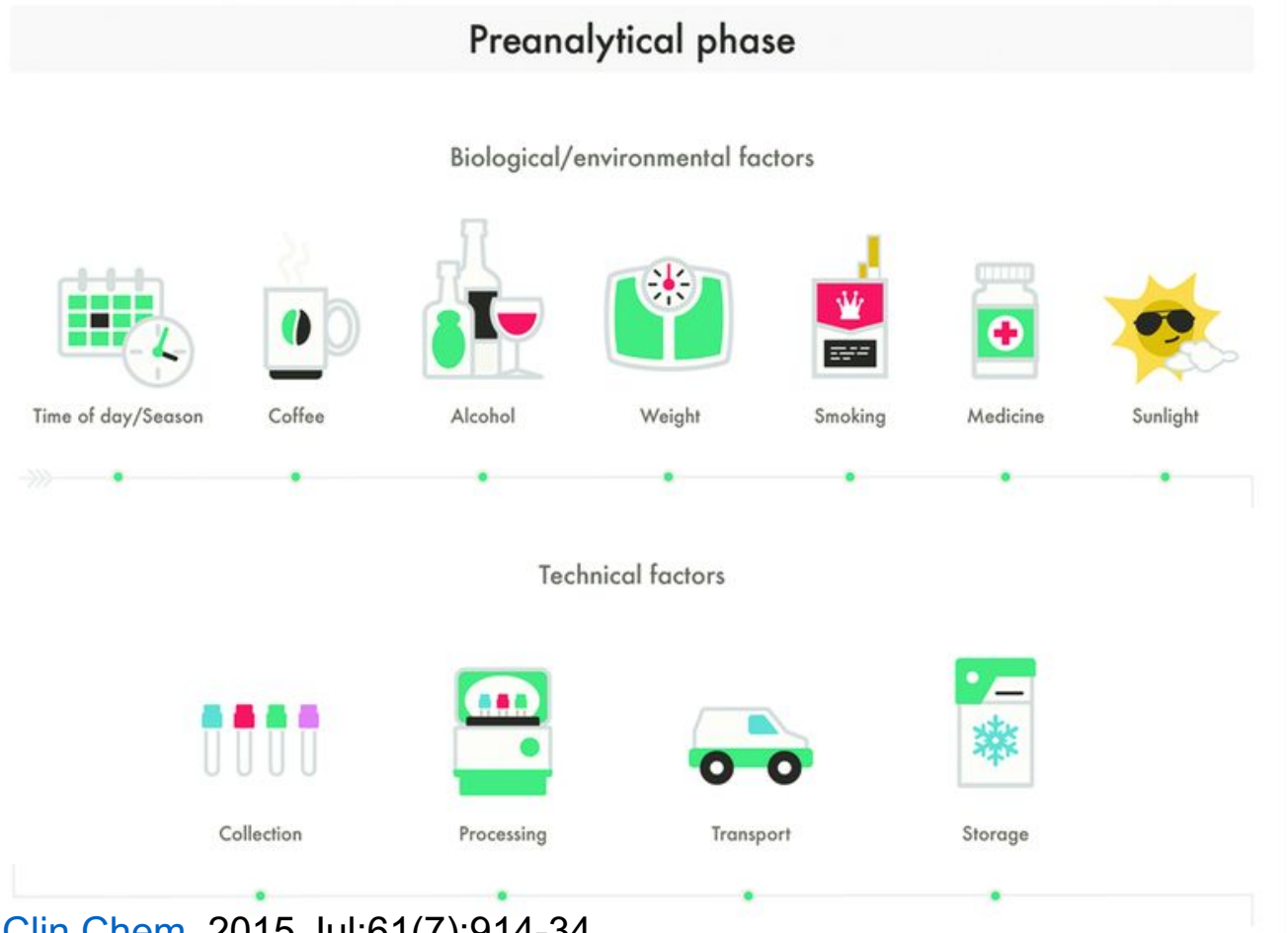
- Physicians rely on accurate laboratory test results for diagnosis and guiding therapy: more than 70% of clinical decisions are based from information derived from laboratory results (MLO Med Lab Obs. 2014 May;46(5):22, 24, 26)
- 10^7 € of funding may be lost each year in clinical trials in the EU due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

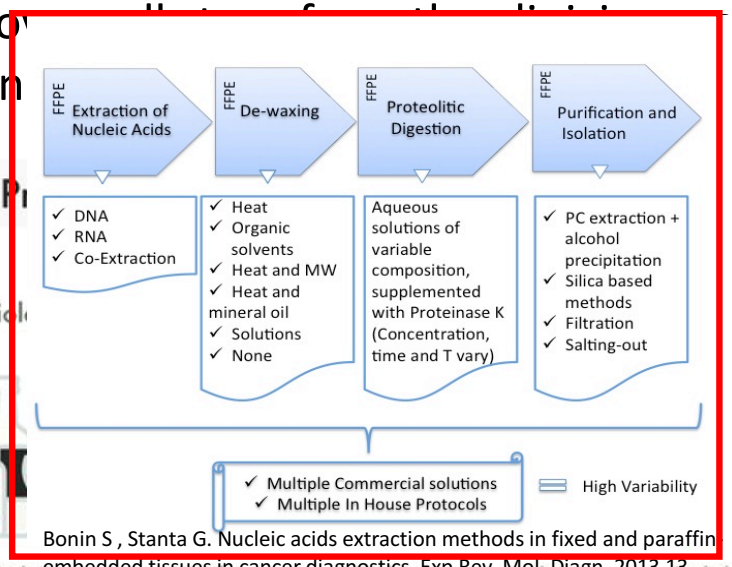
Pre-analytical phase: covers all steps from the clinicians requests to the beginning of the analytical examination, included nucleic acid or protein extractions



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

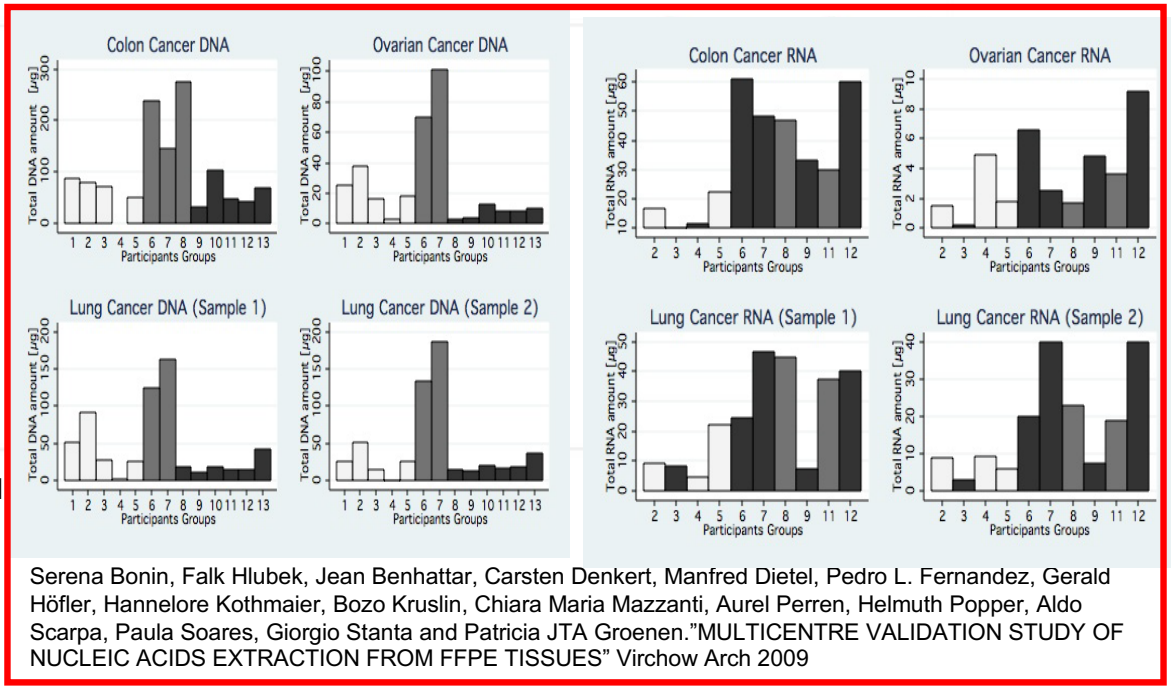
Why extractions into pre-analytics?

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

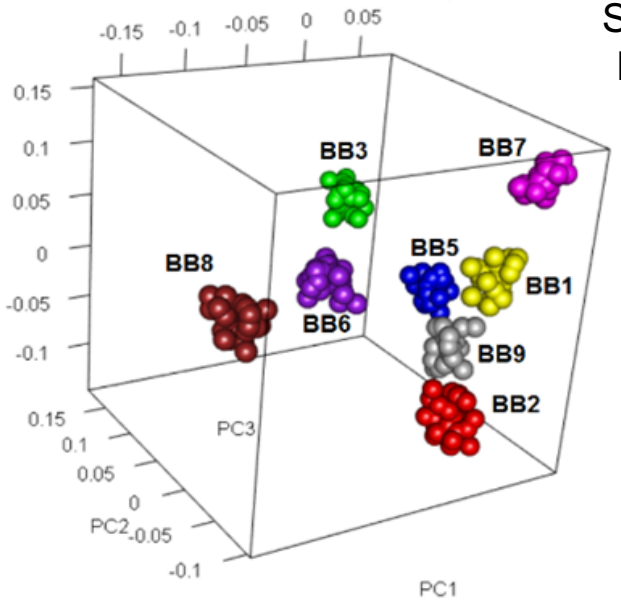
European healthy subjects

EDTA-plasma from 9 biobanks

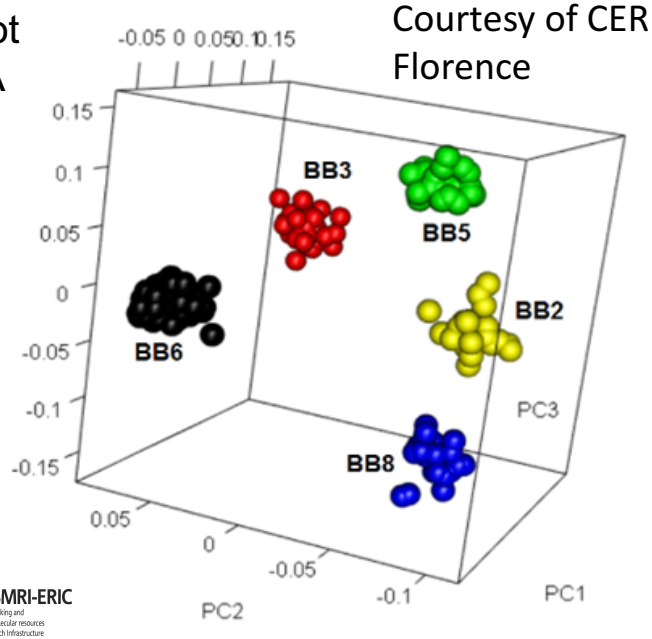
Serum from 5 biobanks

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

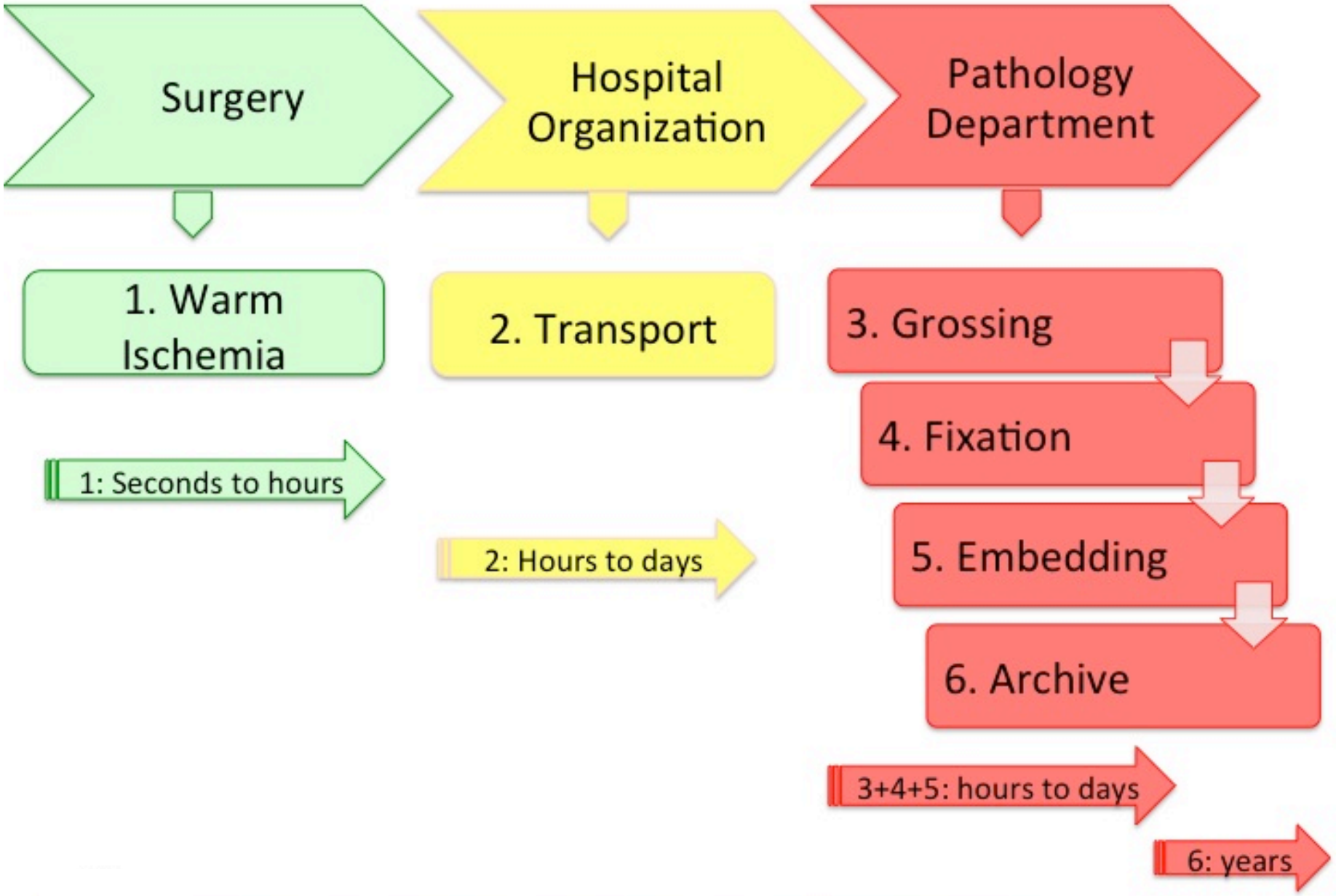
AIMS OF PREANALYTICAL CONDITIONS

Preservation in Archive FFPE Tissues of

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

Preanalytical Conditions

Outside the pathology lab **Inside the pathology lab**



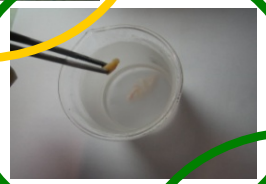
SPIDIA

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

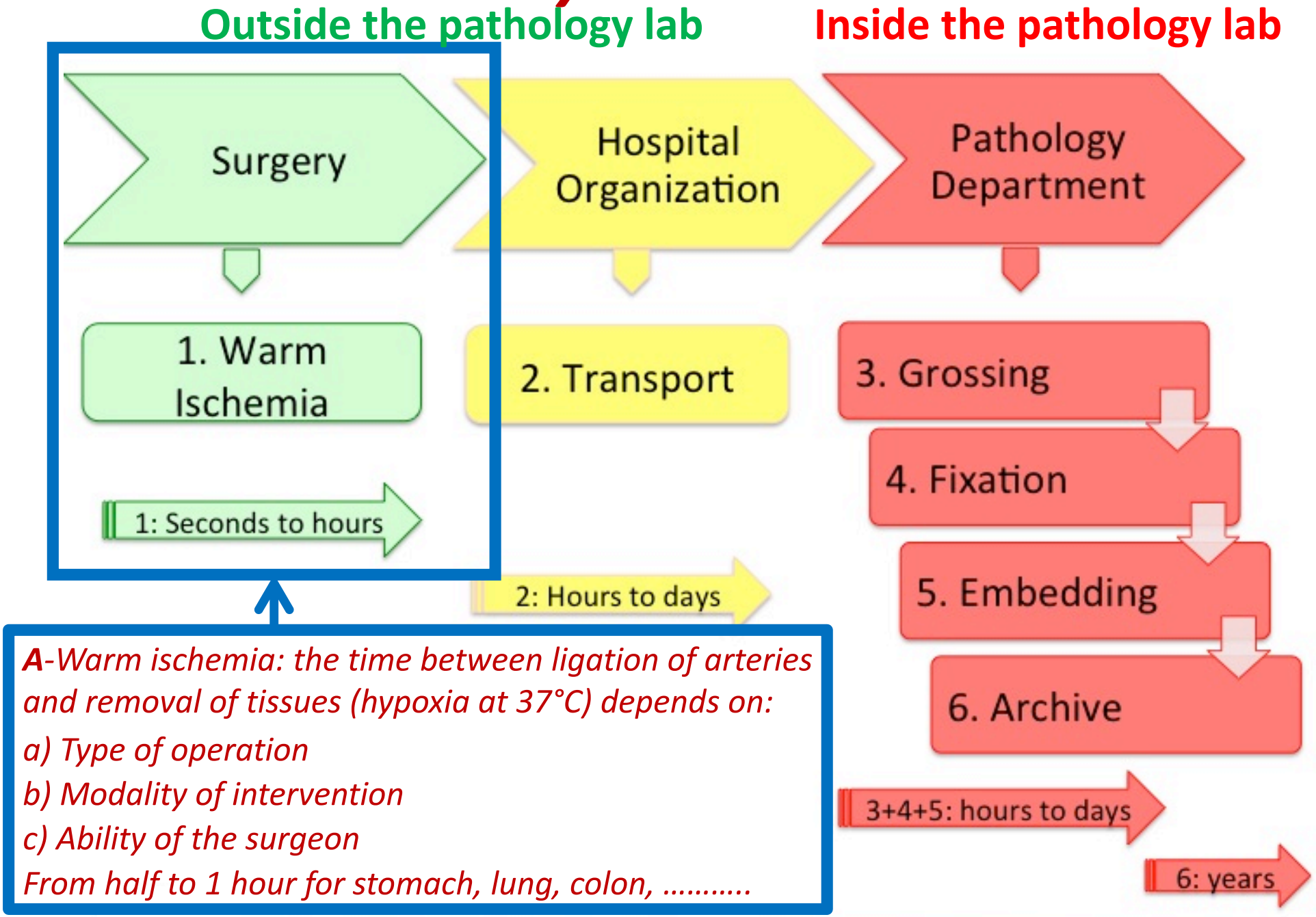
Sample
preparation

Analysis

can be avoided
can be reduced
not avoidable

Original design made available by Kurt Zatloukal

Preanalytical Conditions



Pre-analytical Conditions

Outside the pathology lab

Inside the pathology lab

Surgery

Hospital Organization

Pathology Department

1. Warm Ischemia

2. Transport

3. Grossing

4. Fixation

5. Embedding

6. Archive

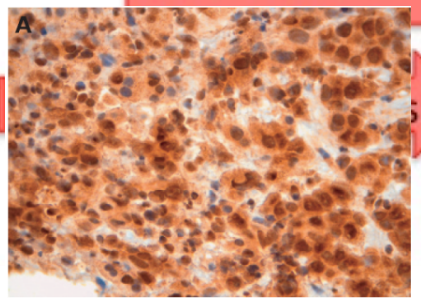
1: Seconds to hours

2: Hours to days

6: years

***B**-In the surgical theatre the use of specific techniques (heat cutting, …) or simply the traction and distortion of tissues can influence gene expression modification, protein coagulation or their diffusion into extracellular spaces with consequences for IHC results.*

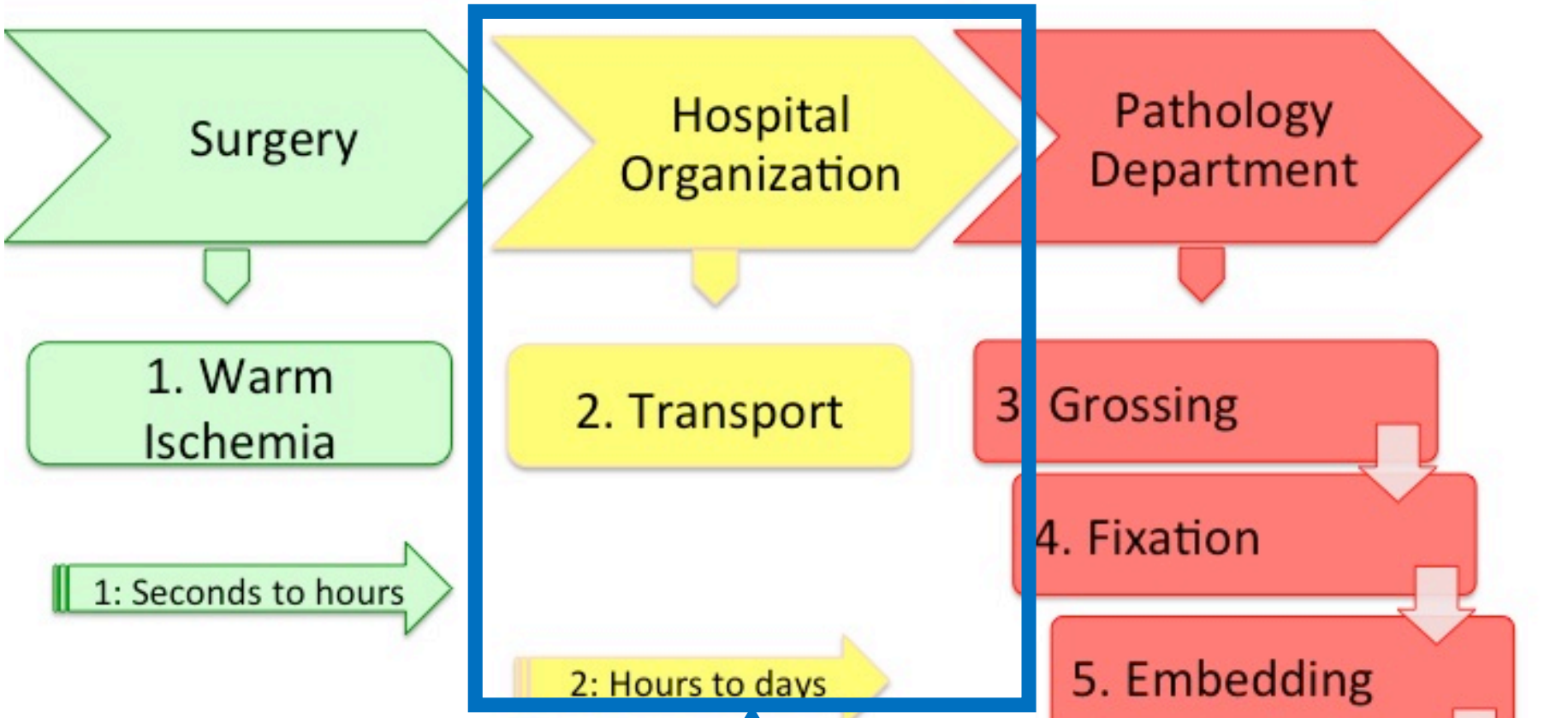
***C**-IHC reliability and RNA analysis can also be affected by common pathological processes such as hemorrhagic diffusion in tissues, necrosis, inflammation or apoptosis.*



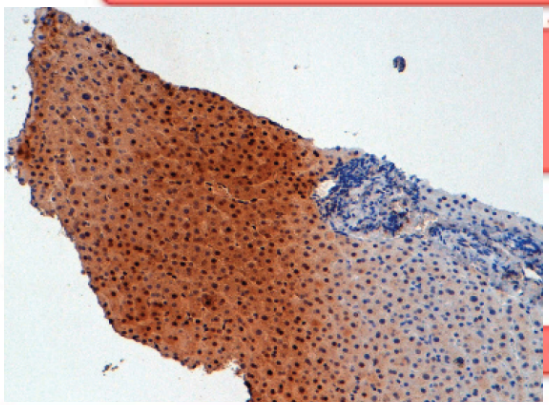
Pre-analytical Conditions

Outside the pathology lab

Inside the pathology lab



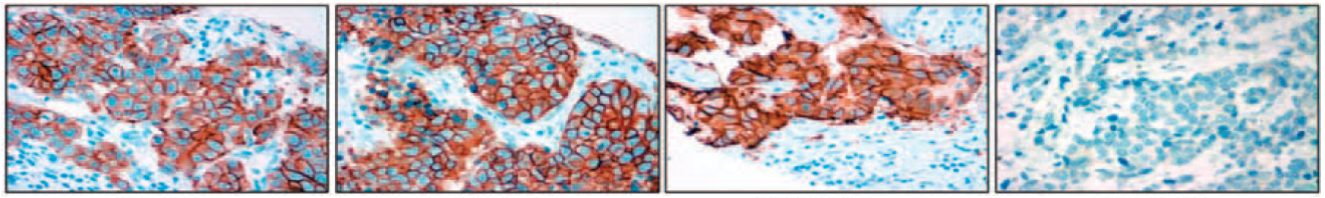
Interval from the surgical table to the pathology lab
Alternatives to prevent cold ischemia:
*a) Tissues left fresh transported at r.t. or in wet ice**
b) Tissues immersed in formalin
*c) Transport at 4°C under vacuum**
Avoid to air dry the tissues



**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

Surgery

Surgical Specimen >2cm.

Under-vacuum (Tissue Safe) — Surgical Theatre

Kept at 4° C till transfer (1-72h)

Tissue Banking (-80° C)

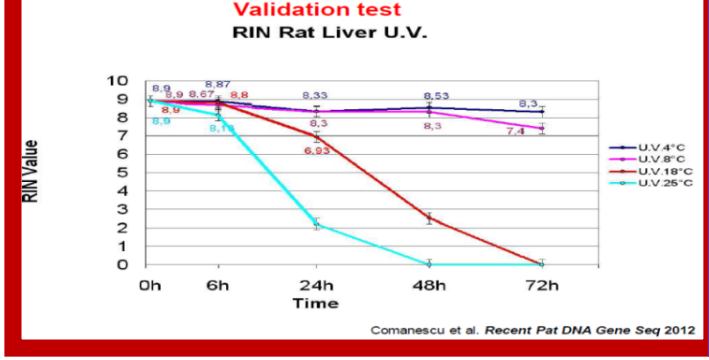
Grossing

P.B. Formalin (12-24h)

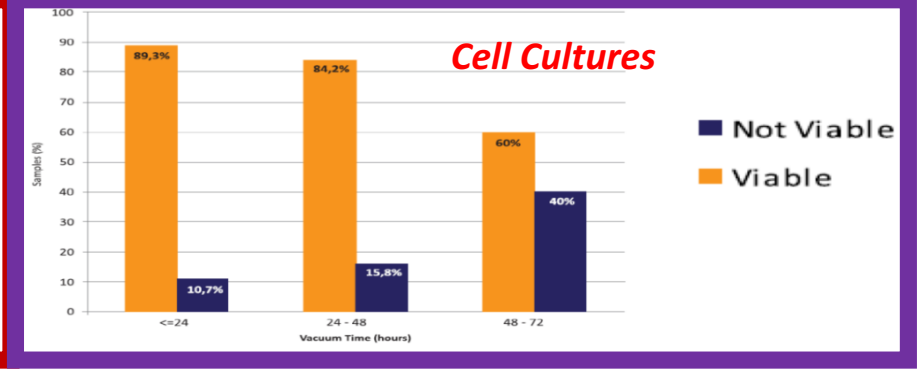
Embedding

Cell coltures — Pathol. Labs.

Bussolati-2014



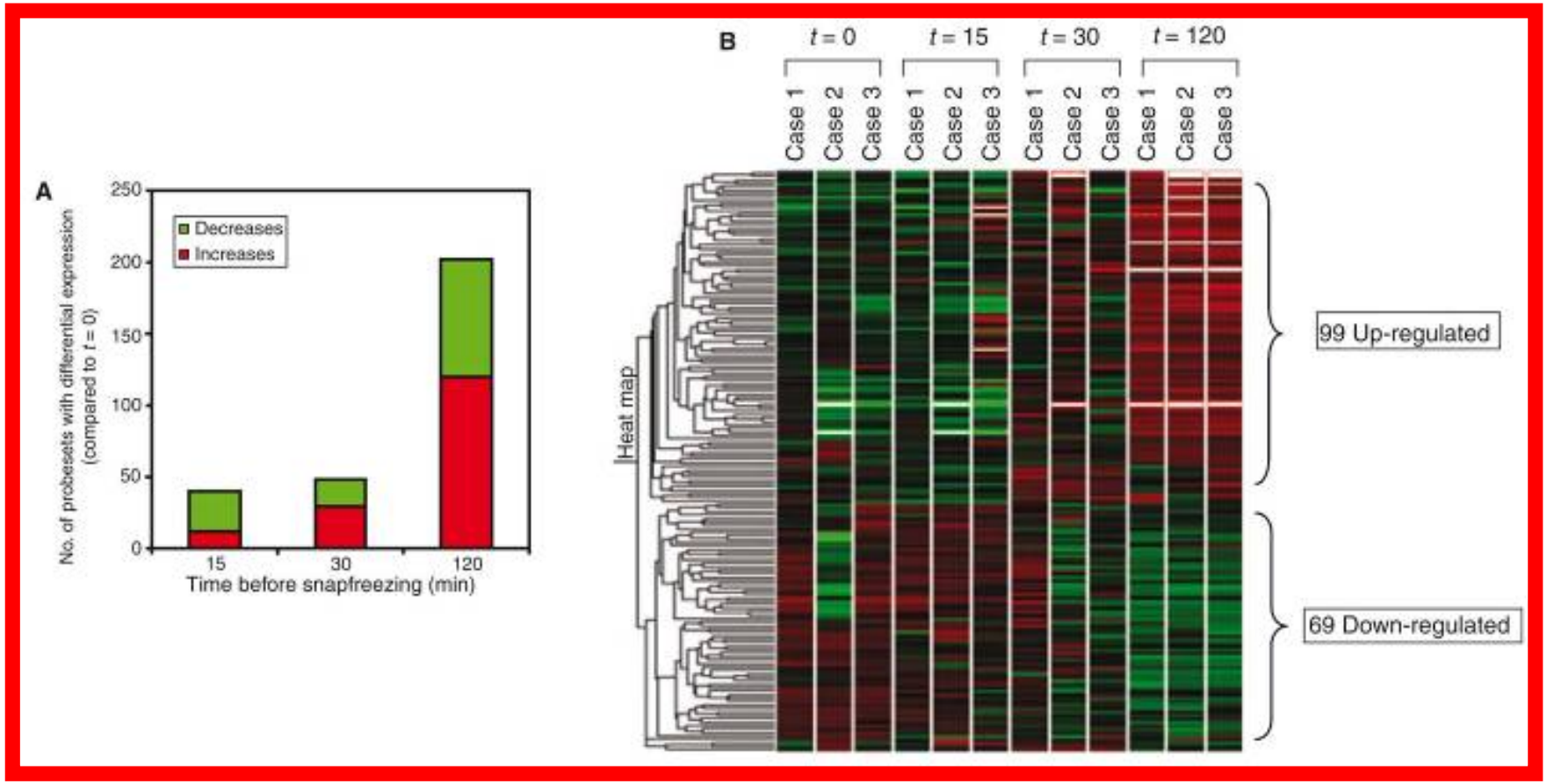
Comanescu et al. *Recent Pat DNA Gene Seq* 2012



G. Bussolati Graz 2014

 # 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.

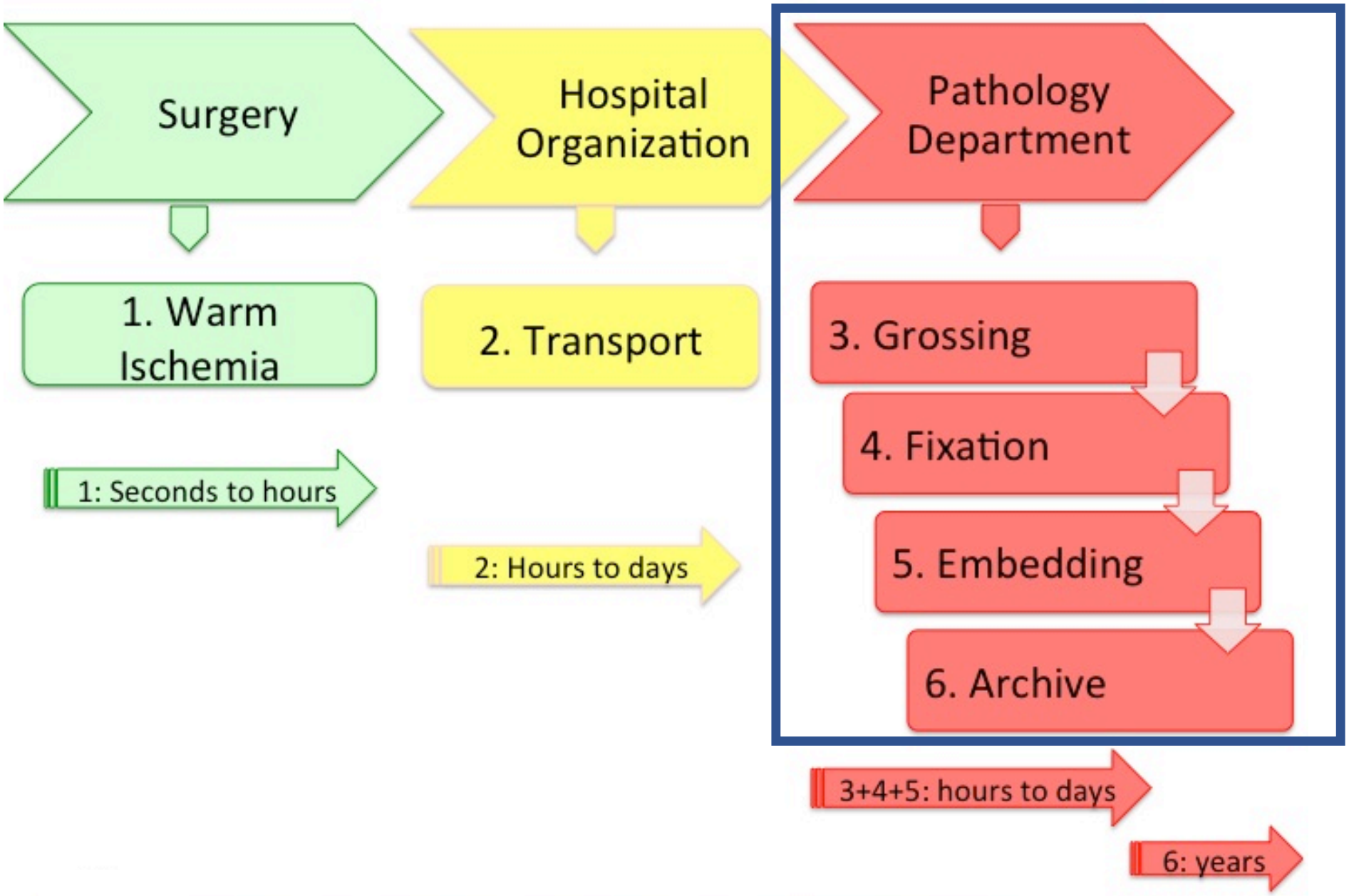


Histopathology 2010, **56**, 240–250. DOI: 10.1111/j.1365-2559.2009.03470.x

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,[1] David A Levison,[1] Robert J C Steele & Neil M Kernohan[1]

Preanalytical Conditions



CLINICAL TISSUES – ARCHIVE TISSUES

DIAGNOSTICS

SURGERY

Surgical Left-over

HUMAN TISSUES DIAGNOSTIC FLOW

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.* $CH_2=O + H_2O \rightleftarrows OH-(CH_2O)-H$

*# 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.*

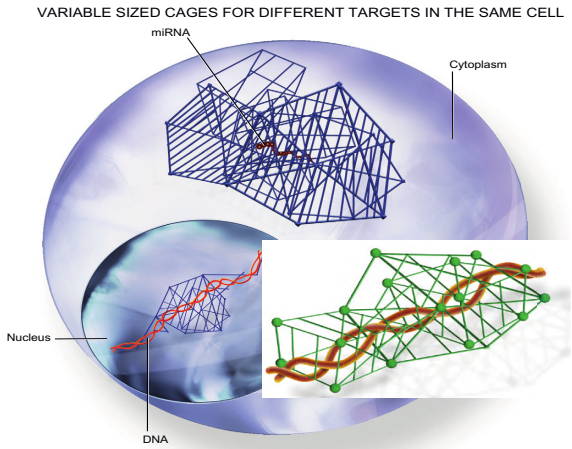
*# Alterations are quantitatively related to **time of fixation**.*

*# **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

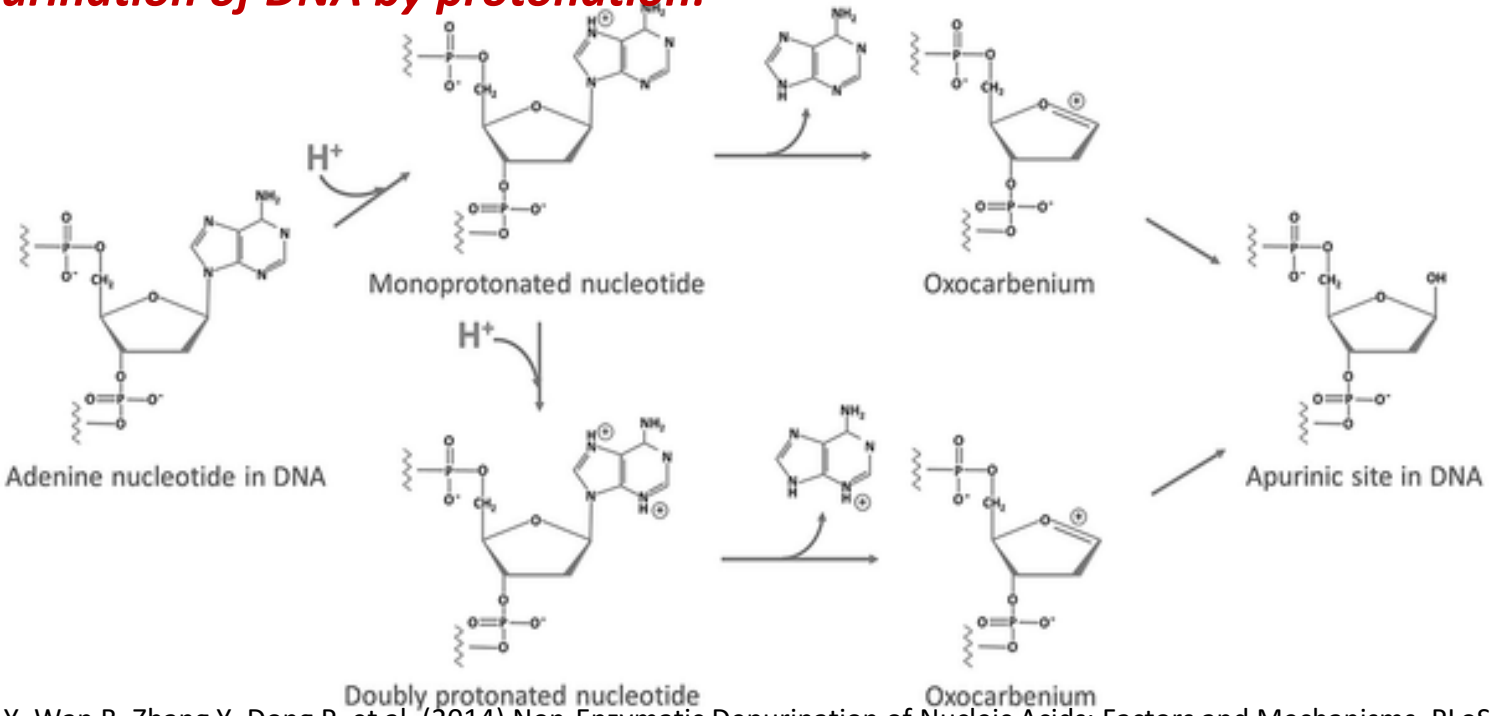
DNA:

1.Mechanical rupture:



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

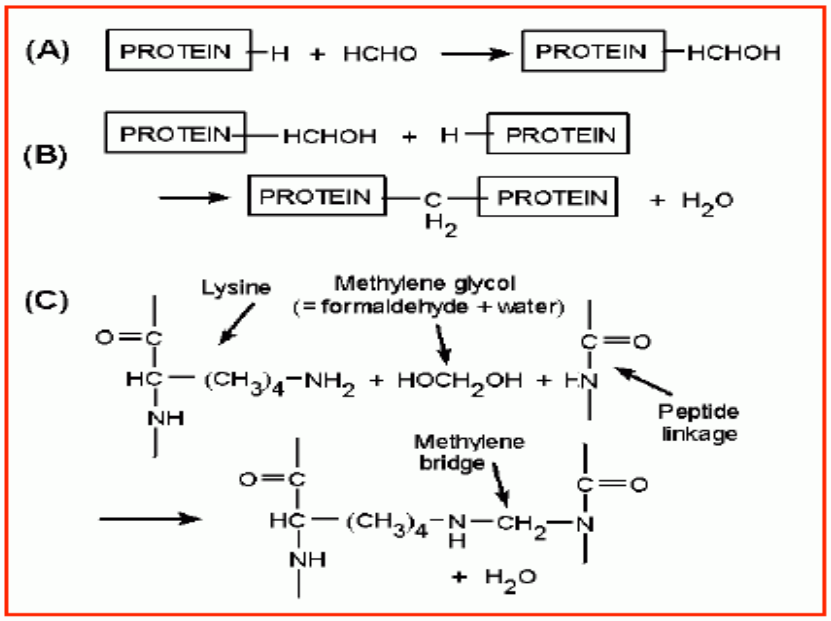
2.Depurination of DNA by protonation:



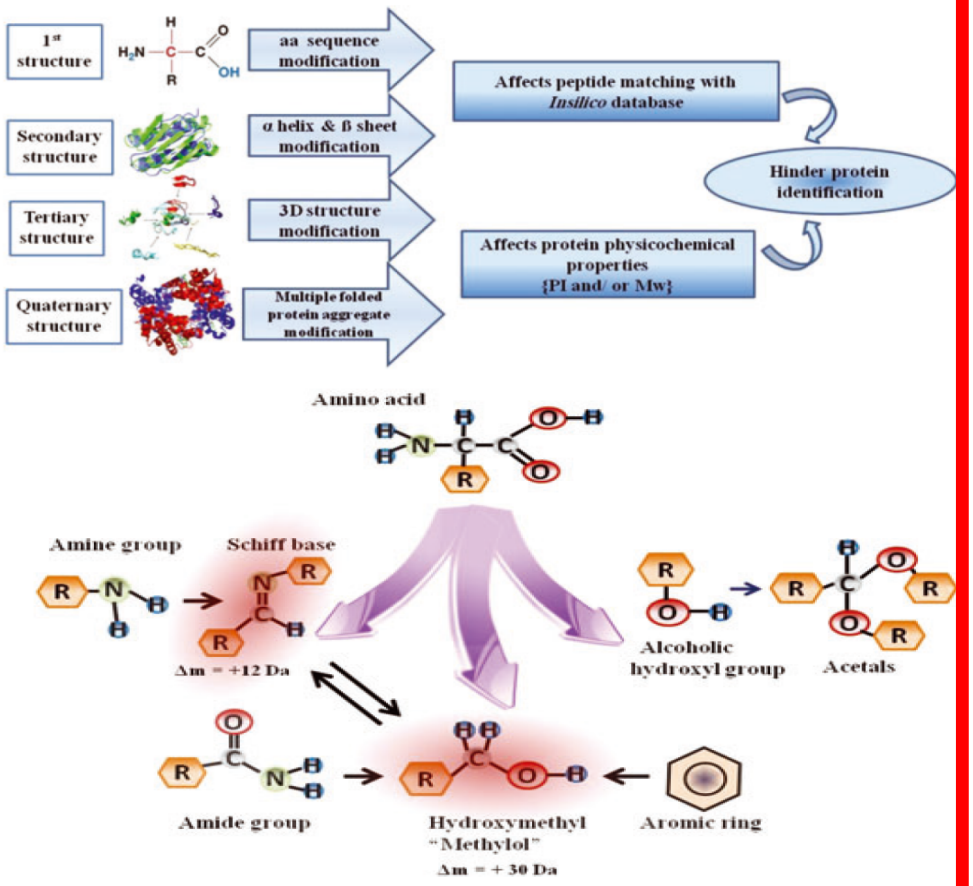
An R, Jia Y, Wan B, Zhang Y, Dong P, et al. (2014) Non-Enzymatic Depurination of Nucleic Acids: Factors and Mechanisms. PLoS ONE 9(12): e115950.

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

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

RNA:

Addition of methylol groups (CH_2-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 bioptic tissues and quantitative RT-PCR reliability". Exp Mol Pathol 87:146–151;2009

QUALITY AND QUANTITY OF DEGRADED RNA

RNA

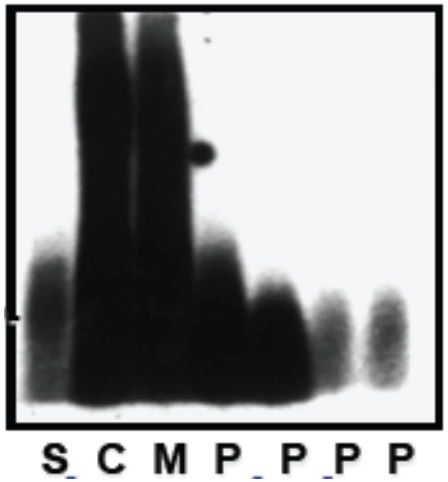
#Formalin: Biopsy < 120 – 200 bases
Autopsy < 70 bases

#Bouin solution Biopsy < 70 bases

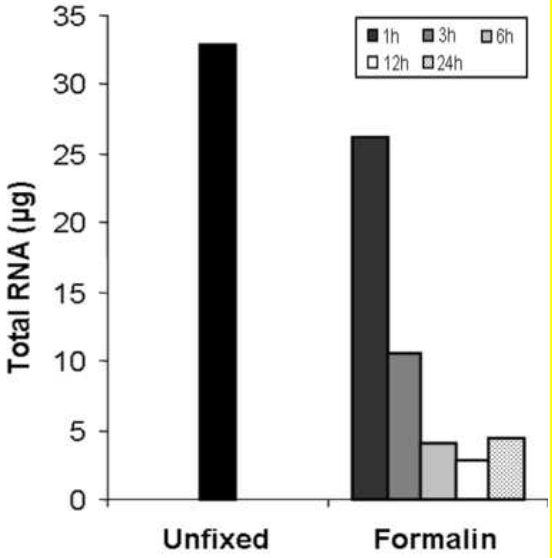
#Alcoholic fix.: Biopsy < about 600 bases

DNA

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

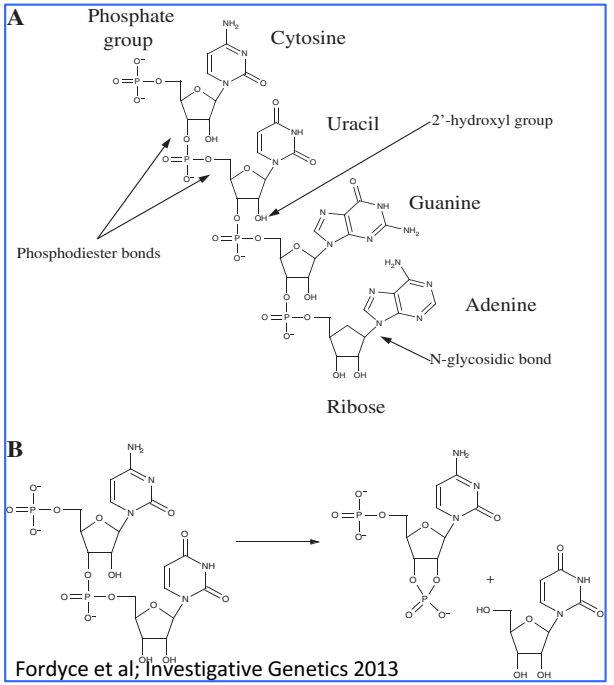


Bonin S., Petrera F., Stanta G. PCR and RT-PCR Analysis In Archival Postmortem Tissues. In Fuchs J, Podda M.Encyclopedia Of Diagnostic Genomics And Proteomics. M. Dekker, New York: 985-988; 2005 "
G. Stanta and C. Schneider, RNA extracted from paraffin-embedded human tissues is amenable to analysis by 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 thermo-dynamically less stable than DNA because the 2'-OH group on the ribose ring promotes the hydrolytic reaction*



Fordyce et al; Investigative Genetics 2013

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 Archivial 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**

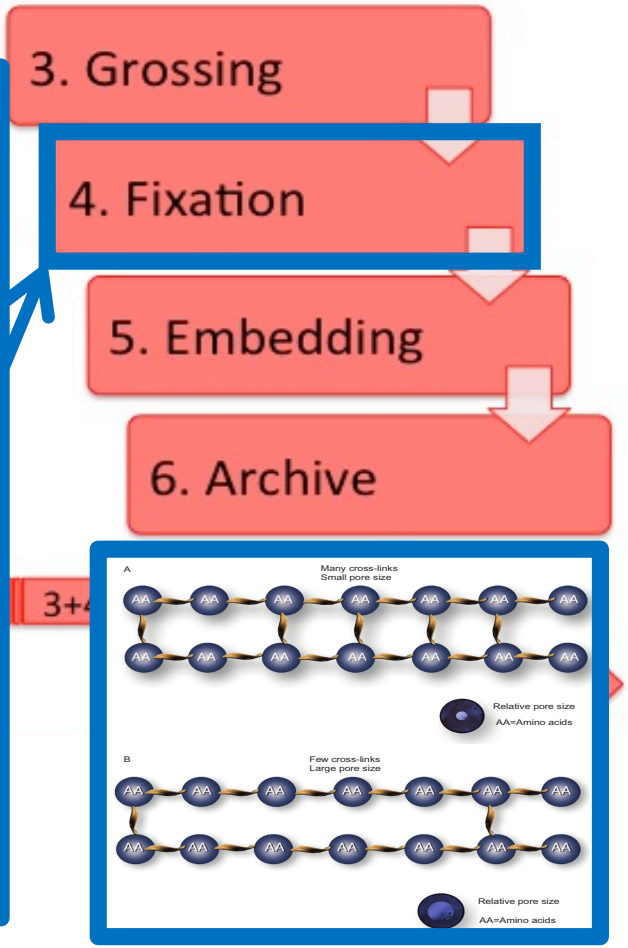
#Standard buffered formalin solution with checked pH, because it can be oxidized to formic acid.

#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.



CEN recommendations for DNA, RNA, proteins extraction from tissues and blood - https://www.cen.eu/Pages/default.aspx

G. Stanta

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 OONE 2013

G. Stanta

Preanalytical Conditions

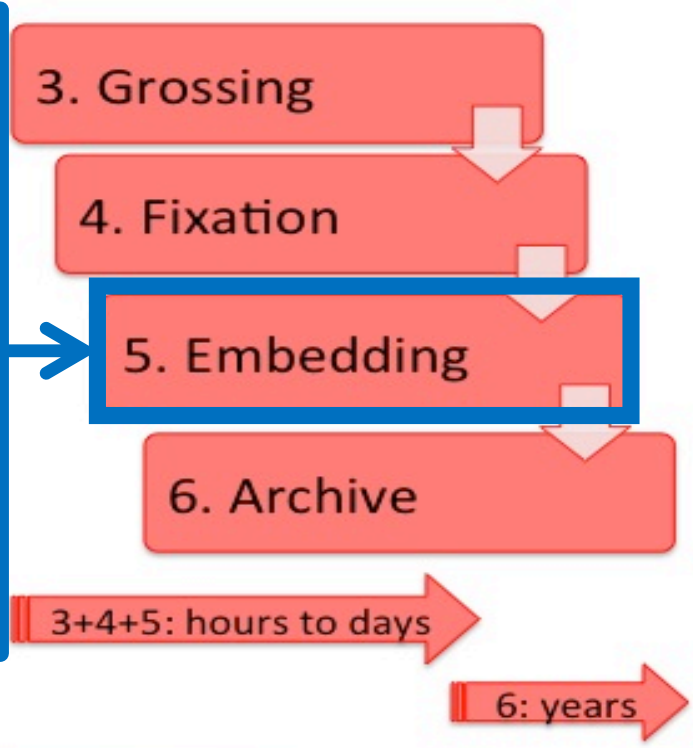
Outside the pathology lab **Inside the pathology lab**

 # 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.



G. Stanta

Preanalytical Conditions

Outside the pathology lab **Inside the pathology lab**

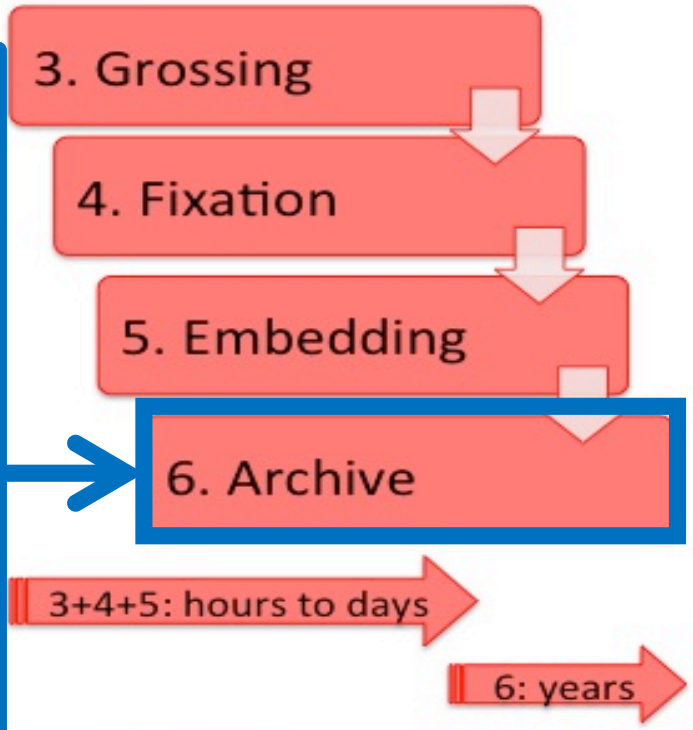
#Storage time may influence the retrieval of antigens and quality of RNA (Balgley, B. (2009) Journal of Proteome Research, 8, 917–925)

#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

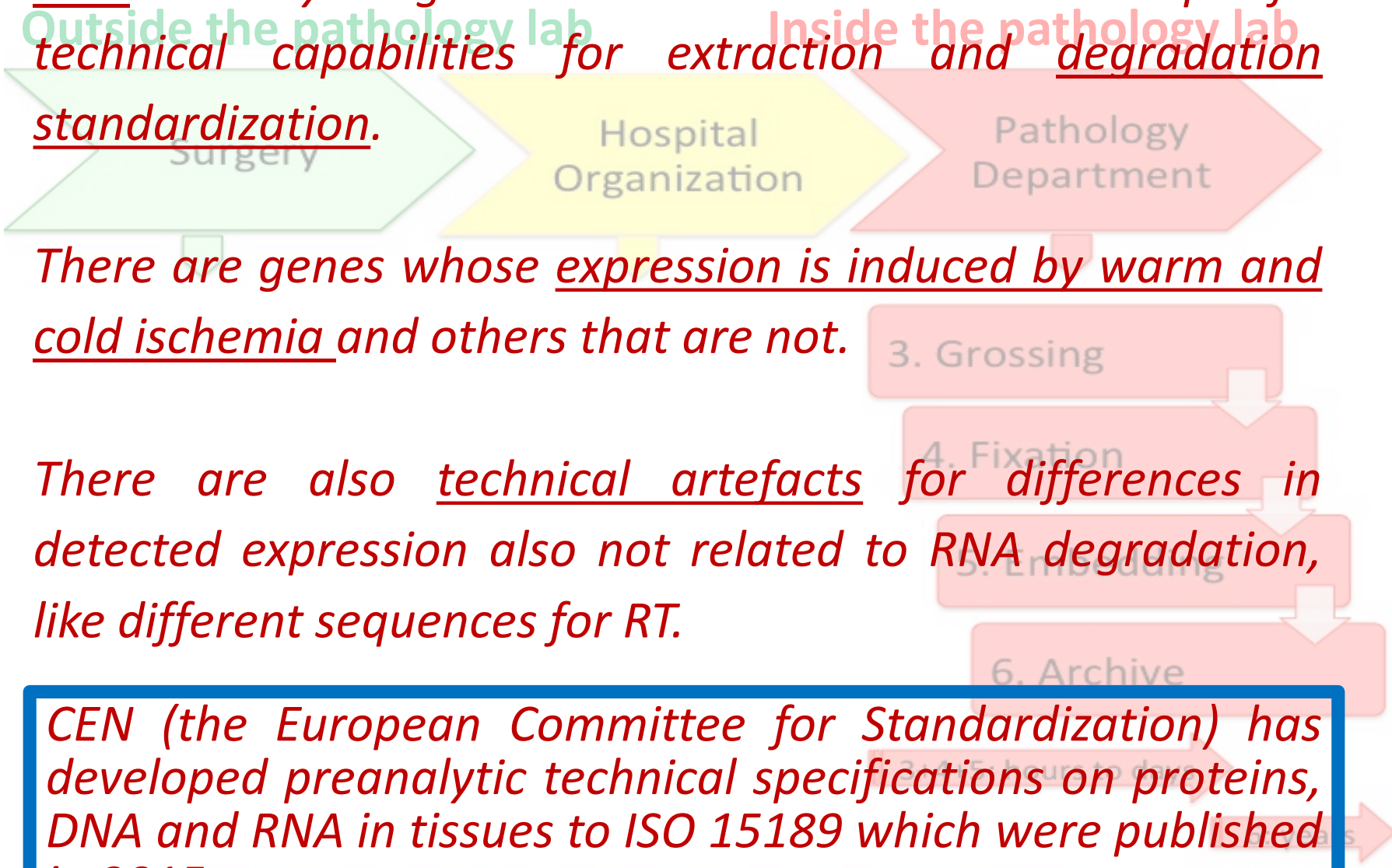
(Thompson, S (2013). Proteomics - Clinical Applications, 7, 241–51)



G. Stanta

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.

G. Stanta

Pre-analytical Conditions in IHC

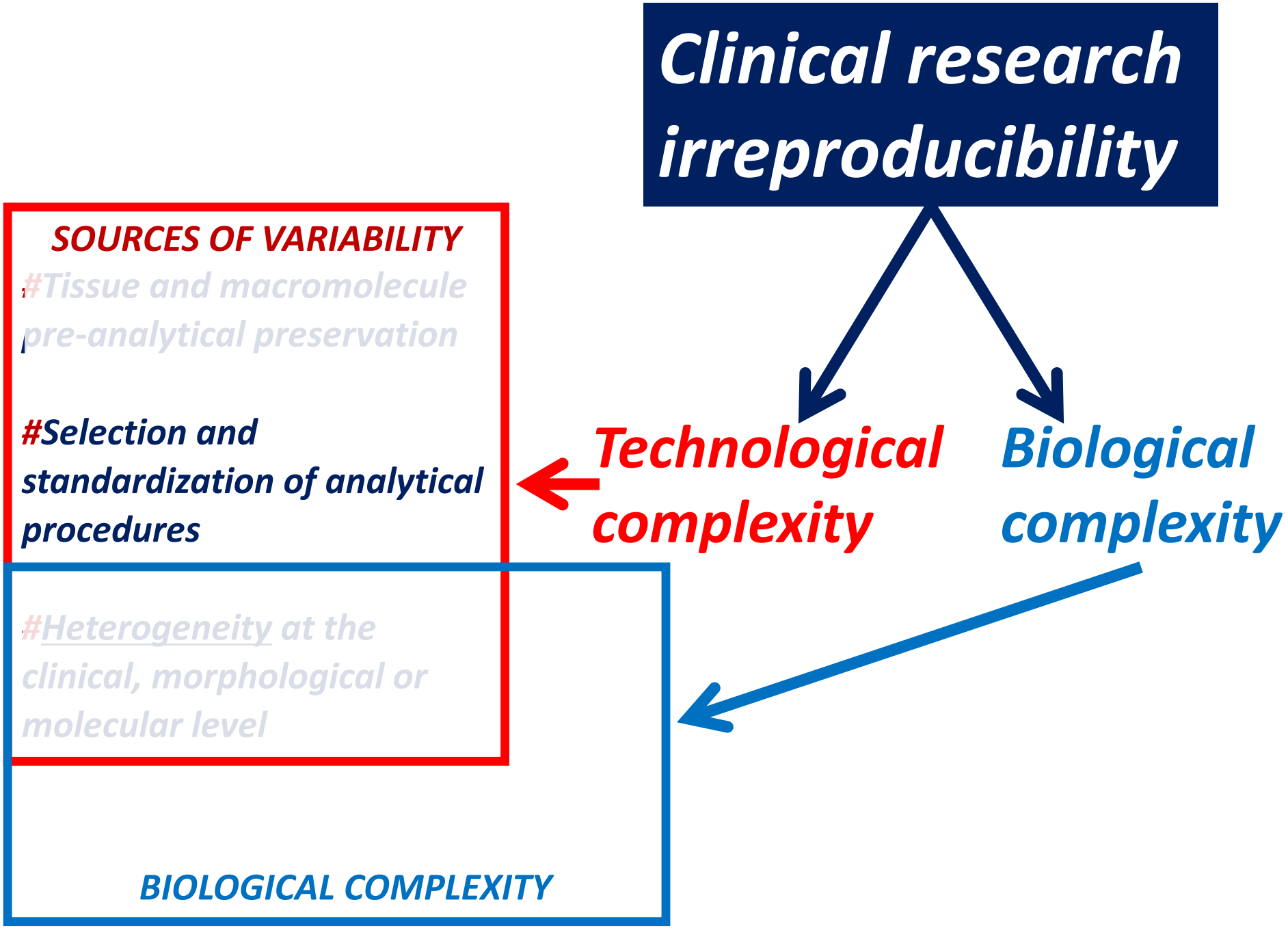
Outside the pathology lab Inside the pathology lab

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

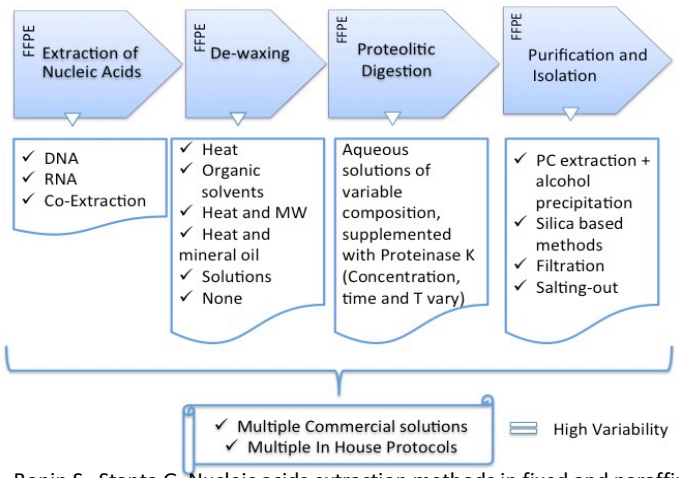
(1) predictable stable;
(2) predictable unstable;
(3) unpredictable.

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
SOURCES OF VARIABILITY
#Tissue and macromolecule pre-analytical preservation
#Selection and standardization of analytical procedures
#Heterogeneity at the clinical, morphological or molecular level
Technological complexity
Biological complexity
BIOLOGICAL COMPLEXITY

G. Stanta



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

SOURCES OF VARIABILITY

#Selection and standardization of analytical procedures

Scant information on the myriad kits and reagents purchased by labs can lead researchers to do inappropriate experiments inadvertently.

A recipe for disaster

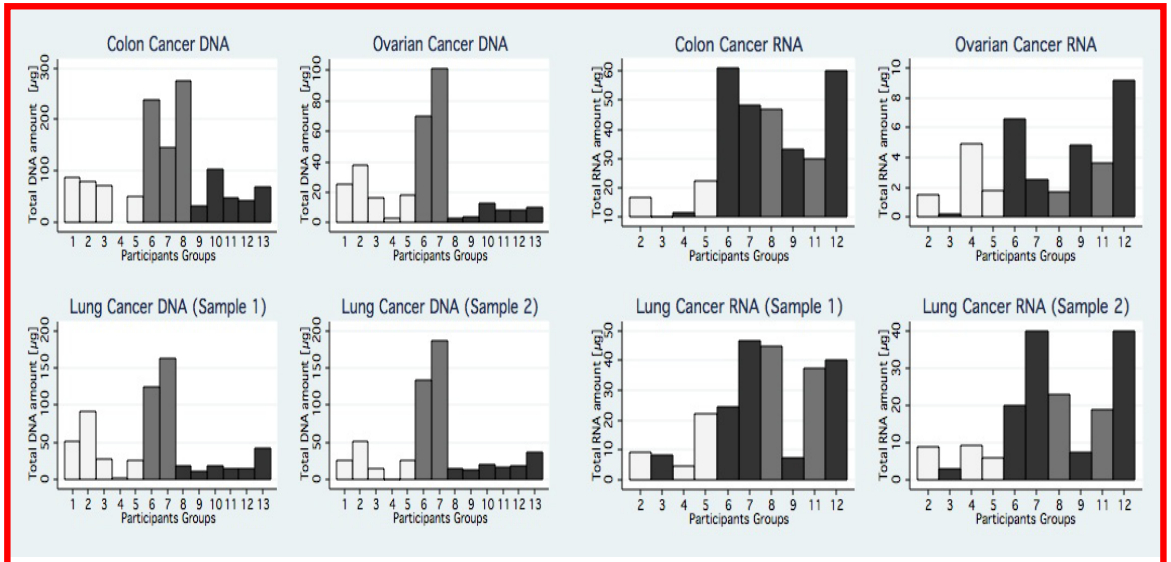
Manufacturers of commercial reagents should follow scientific norms and be open about the ingredients of their products, says **Anna Git**.

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 chemically 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 fortuitously 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 practices of scientific publication. 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 ▸



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

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

Ermanno Nardon [a,b], Marisa Donada [b], Serena Bonin [a,b], Isabella Dotti [a], Giorgio Stanta [a,b,*]

[a] Department of Clinical, Morphological and Technological Sciences, University of Trieste, Italy
[b] International Centre for Genetic Engineering and Biotechnology, Padriciano, Trieste, Italy

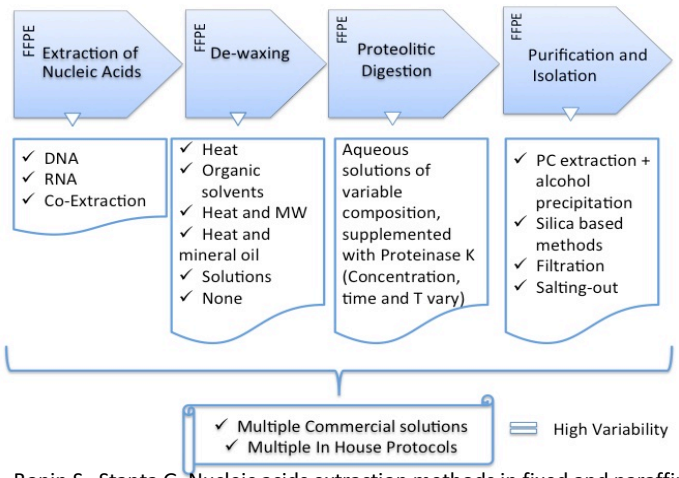
"Reverse transcription yield, indeed, can vary up to 100-fold depending on priming strategy, on the used enzyme, on the starting quantity of target RNA and even on the type of sequence that is going to be detected."

Table 4
Summary of case study results.

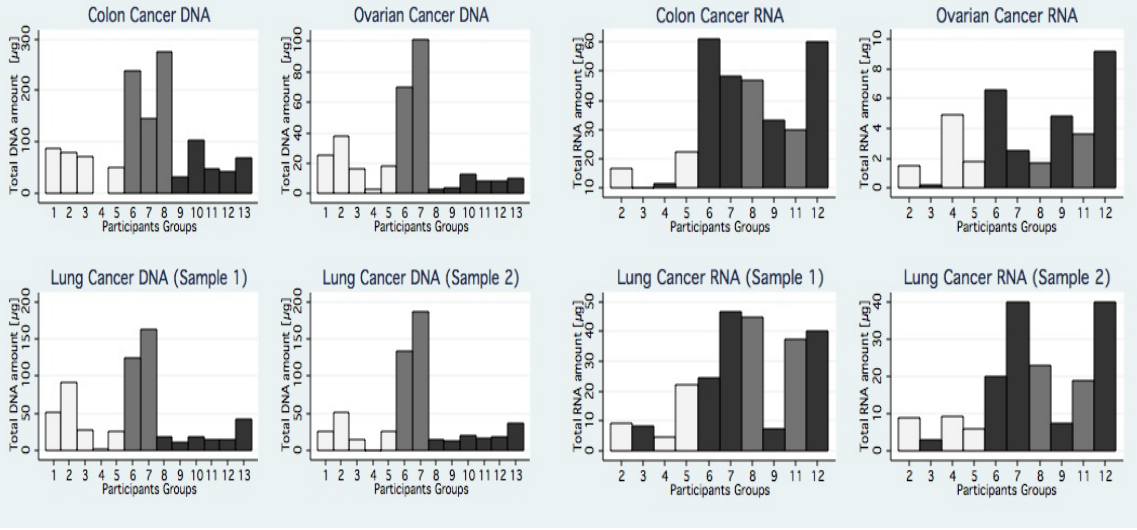
	ACTB gene			TS gene		
	Mean C_t	SD	Min.–Max.	Mean C_t	SD	Min.–Max.
0.14 nmol random hexamers RT	23.45	0.79	22.58–24.94	34.34	0.65	33.30–35.26
3.35 nmol random hexamers RT	21.75	0.78	20.60–23.43	32.16	0.73	30.69–33.24
Difference between matched pairs[a]	1.69	0.26	1.33–2.08	2.18	0.28	1.77–2.71

Case study samples were reverse transcribed in two different conditions and TS and ACTB genes were qRT-PCR amplified. Mean C_t is the mean C_t value of the 12 samples and SD its standard deviation. Min.–Max. is the range of the observed C_ts.
[a] Value obtained subtracting the C_t of a sample submitted to RT with the lower primer concentration from the C_t of the same sample submitted to RT with the higher primer concentration.

G. Stanta



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



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SOURCES OF VARIABILITY

#Selection and standardization of analytical procedures

A reci

Manufacturers of comme
be open about the ingredients of their products, says Anna Gil.

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 chemically 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 fortuitously 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 practices of scientific publication. 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 ▸

26 APRIL 2012 | VOL 484 | NATURE | 435

SOPs-IQCs!!!

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cDNA

priming
ven on

Summary of case study results.

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G. Stanta

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

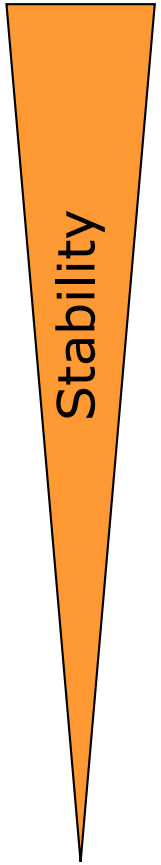
Arch Pathol Lab Med—Vol 138, November 2014

Sample variables

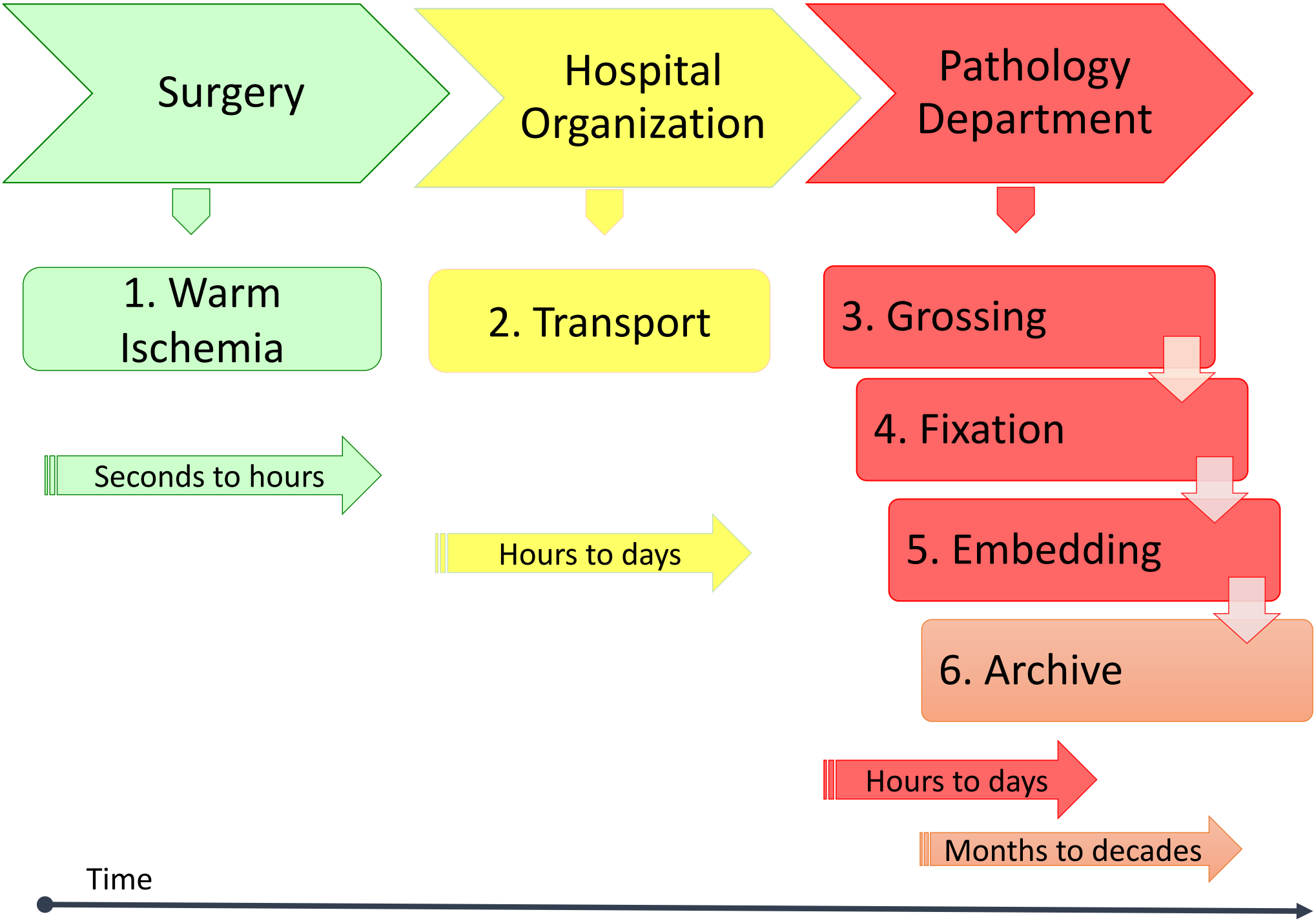
- Tissue type (organ)
- Diseased/normal
- Sample type (biopsy/surgery)
- Peri-operative effects
- Ischemia
- Processing
- Fixation
- Storage
- Analysis

Readout

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



Original design made available by K.urt 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→ 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 **CEN/TS 16827-1**

SPÉCIFICATION TECHNIQUE

TECHNISCHE SPEZIFIKATION 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 **CEN/TS 16827-2**

SPÉCIFICATION TECHNIQUE

TECHNISCHE SPEZIFIKATION 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 **CEN/TS 16827-3**

SPÉCIFICATION TECHNIQUE

TECHNISCHE SPEZIFIKATION 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

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COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

CEN/TS- European Technical Specification for FFPE tissues

Contents

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

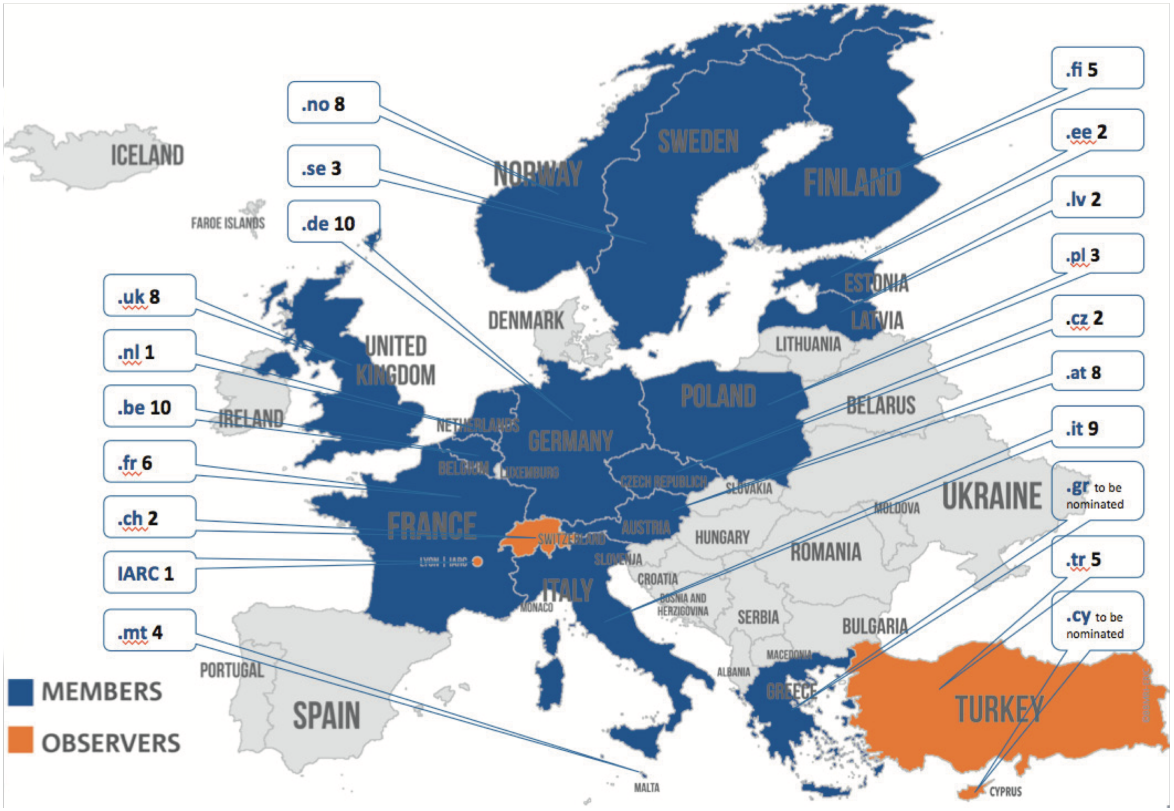


Image made available by Giorgio Stanta

BBMRI-ERIC Self assessment Survey

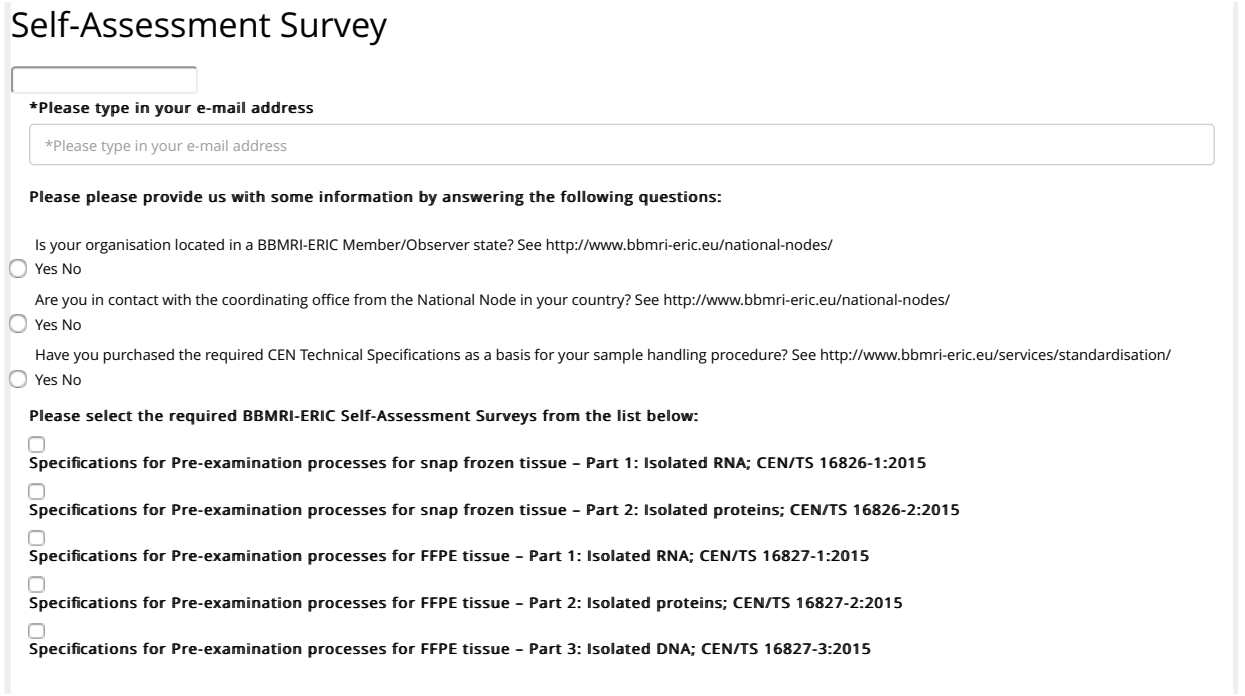
Registration

Compliance Assessment

Model 1: Biobank internal use

Model 2: Biobank submits report to BBRI-ERIC

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



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

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

SPIDIA4P

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)

SPIDIA4P

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