«Effects of routine pathologic procedures on nucleic acid and proteins»

Istanbul 19-21 September 2013

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Dipartimento di Scienze Mediche
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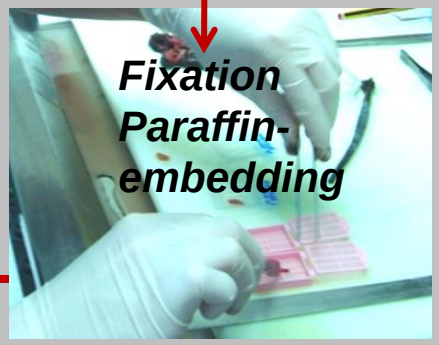
DIAGNOSTICS

SURGERY

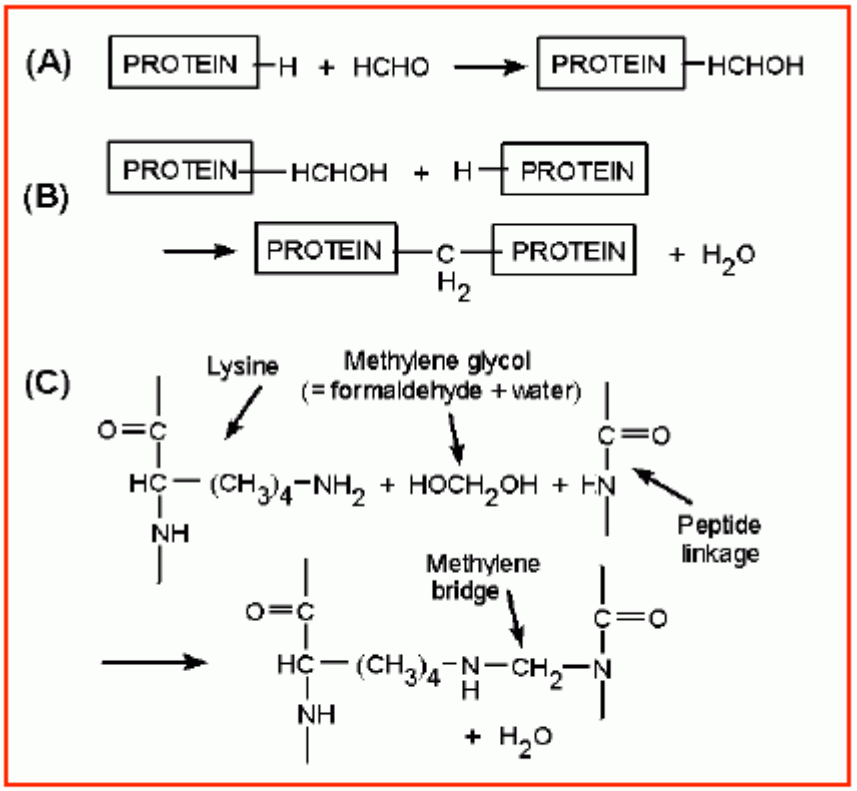
HUMAN TISSUES DIAGNOSTIC FLOW



Surgical Left-over

*#**Millions of residual human tissue specimens** with any kind of even rare diseases are stored often for decades in the archives of hospitals.*
*#These tissues are **related to clinical records** and very often to very developed health informatic systems with follow-up and outcome information.*
*#The archives are run by pathologists that have access to all the information and are bound by **professional secrecy**.*

FORMALIN DAMAGE



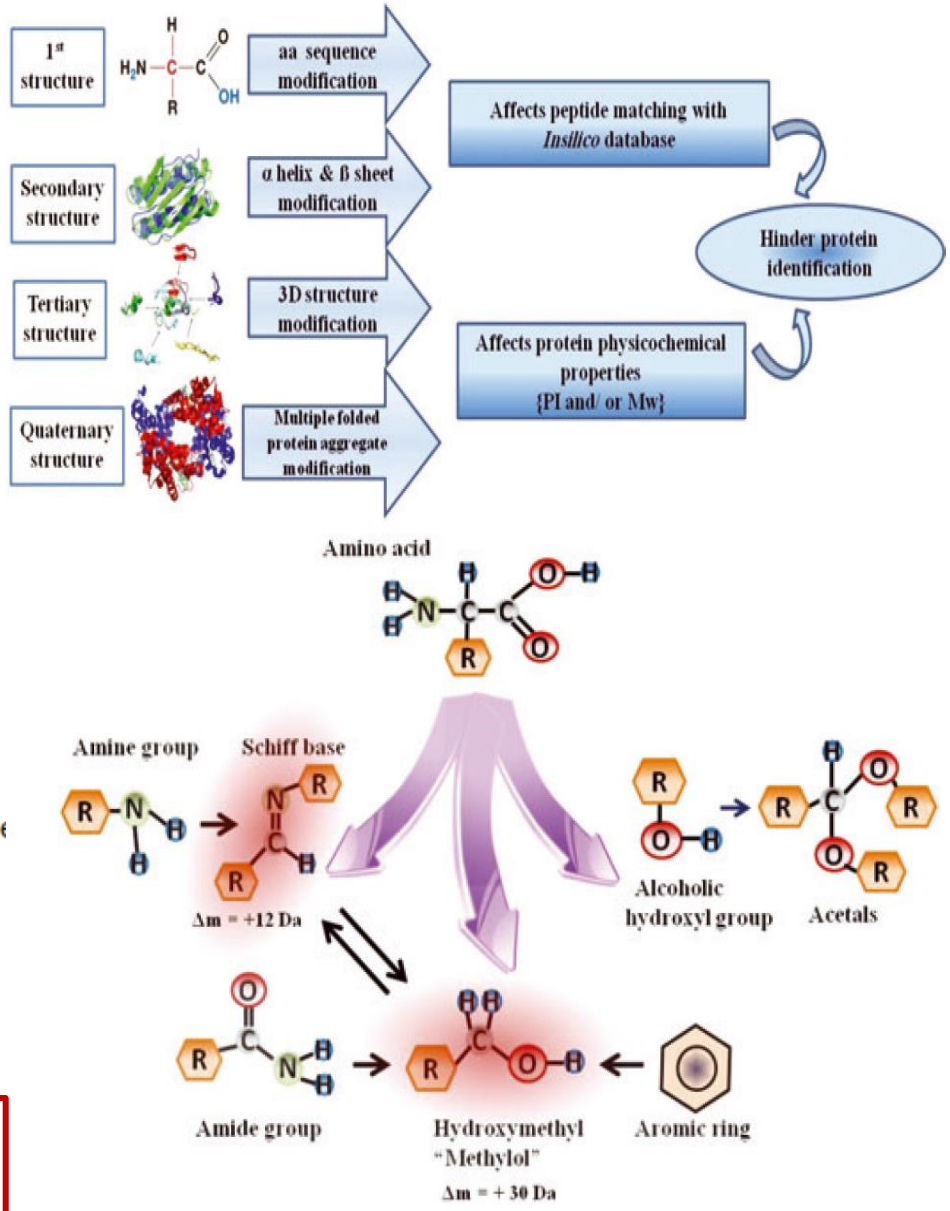
Kiernan,JA. Formaldehyde, formalin, paraformaldehyde and glutaraldehyde: What they are and what they do. Microscopy Today 00-1 pp. 8-12 (2000).

-Addition of formaldehyde molecules to proteins

-Methylene bridge formation between proteins

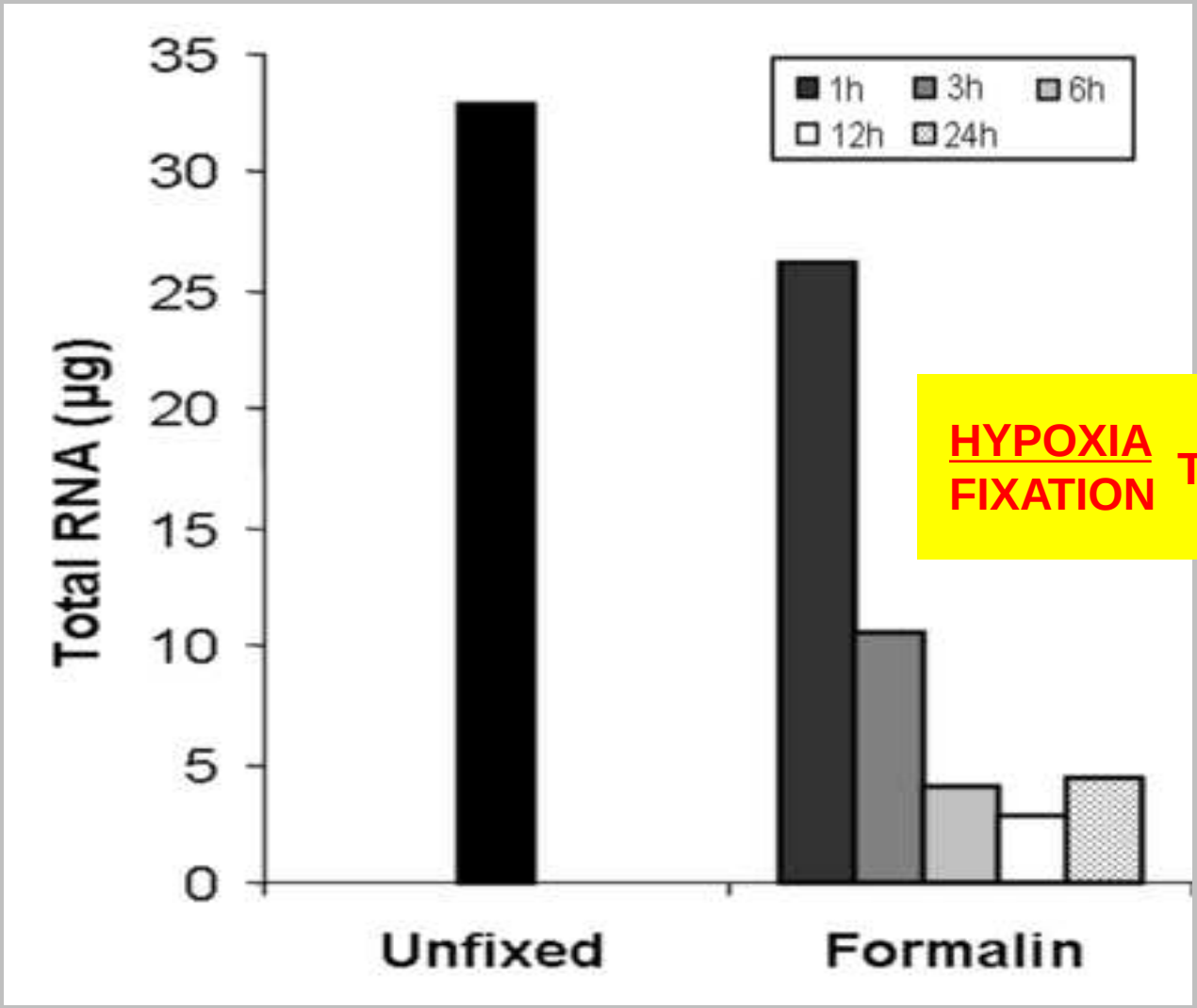
-Cross binding between lysine and methylene

Addition of methylol g. (CH_2-OH) during formalin fixation to bases makes RNA resistent to RT. All the 4 bases show this type of alteration but to a different level (40% A ÷ 4% U).
>>> RNA Demodification (20 min in TE buffer at 70°C)



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

RNA DEGRADATION IN FORMALIN-FIXED CELLS BY FIXATION TIME



HYPOXIA
FIXATION **TIME RATIO**

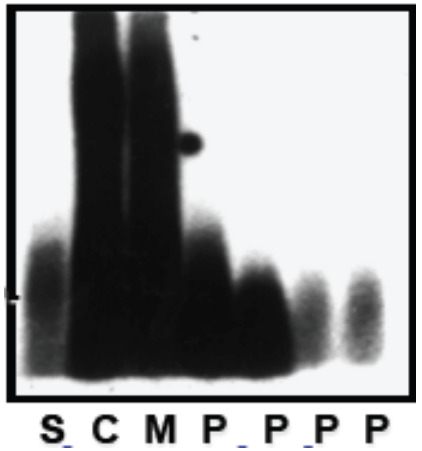
I.Dotti, S.Bonin, G. Basili, E. Nardon, A. Balani, S. Siracusano, F. Zanconati, S. Palmisano, N. De Manzini and G. Stanta. "Effects of formalin, methacarn and FineFIX fixatives on RNA preservation". Diagn Mol Pathol 19:112-122; 2010

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

QUALITY OF EXTRACTED NUCLEIC ACIDS

RNA

#Formalin:	Biopsy < 120 – 200 bases Autopsy < 70 bases
#Bouin solution	Biopsy < 70 bases
#Alcoholic fixatives	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."

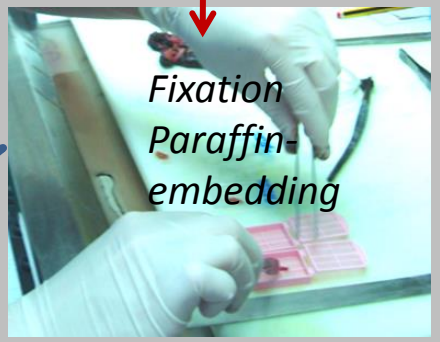
FORMALIN BANNING
NEW FIXATIVES

	Fresh T	Blood	Fixed T.	New Fixative
DNA	High quality	High quality	Degraded Sequence alteration	High quality
RNA	High quality	High quality	Degraded < 70 bb efficiency	High quality
PROTEIN	High quality	High quality	Methylenic bonds	High quality

DIAGNOSTICS

SURGERY

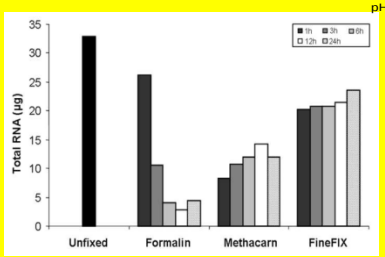
HUMAN TISSUES DIAGNOSTIC FLOW

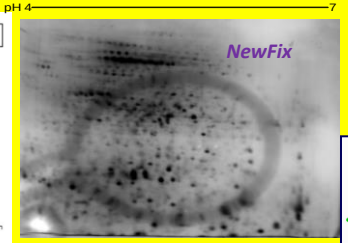


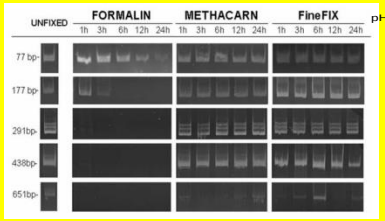
TISSUE FIXATION

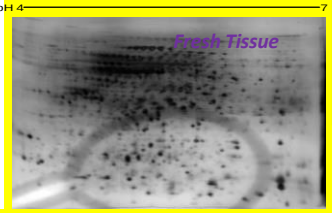
Standardized time of fixation

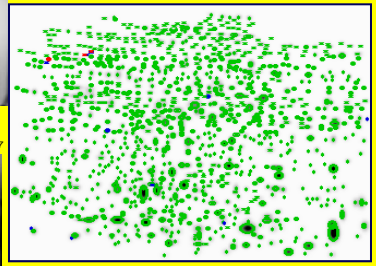
NEW FORMALIN-FREE FIXATIVES











Stanta G, et al.. Diagn Mol Pathol. 2006 ;15:115-23.

SOURCES OF CLINICAL RESEARCH AND DIAGNOSTICS VARIABILITY IN AT

#Tissue and macromolecule pre-analytical preservation

#Heterogeneity at the clinical, morphological or molecular level

#Selection and standardization of analytical procedures

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#Tissue and macromolecule pre-analytical preservation

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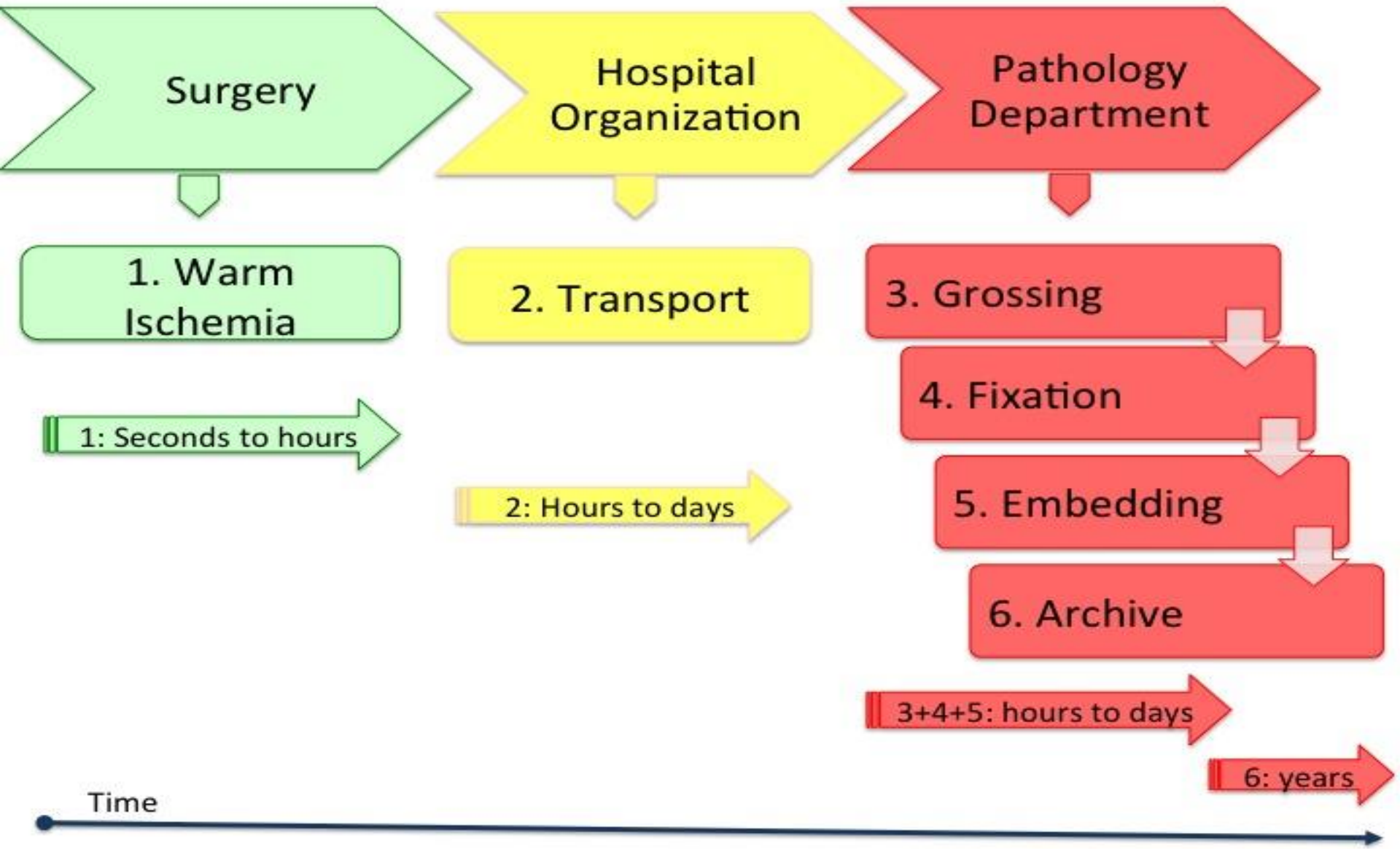
ALL PREANALYTICAL FACTORS AFFECT RNA INTEGRITY MORE SEVERELY THAN DNA



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

Tissue SAFE Vacuum Unit

Dedicated vacuum unit

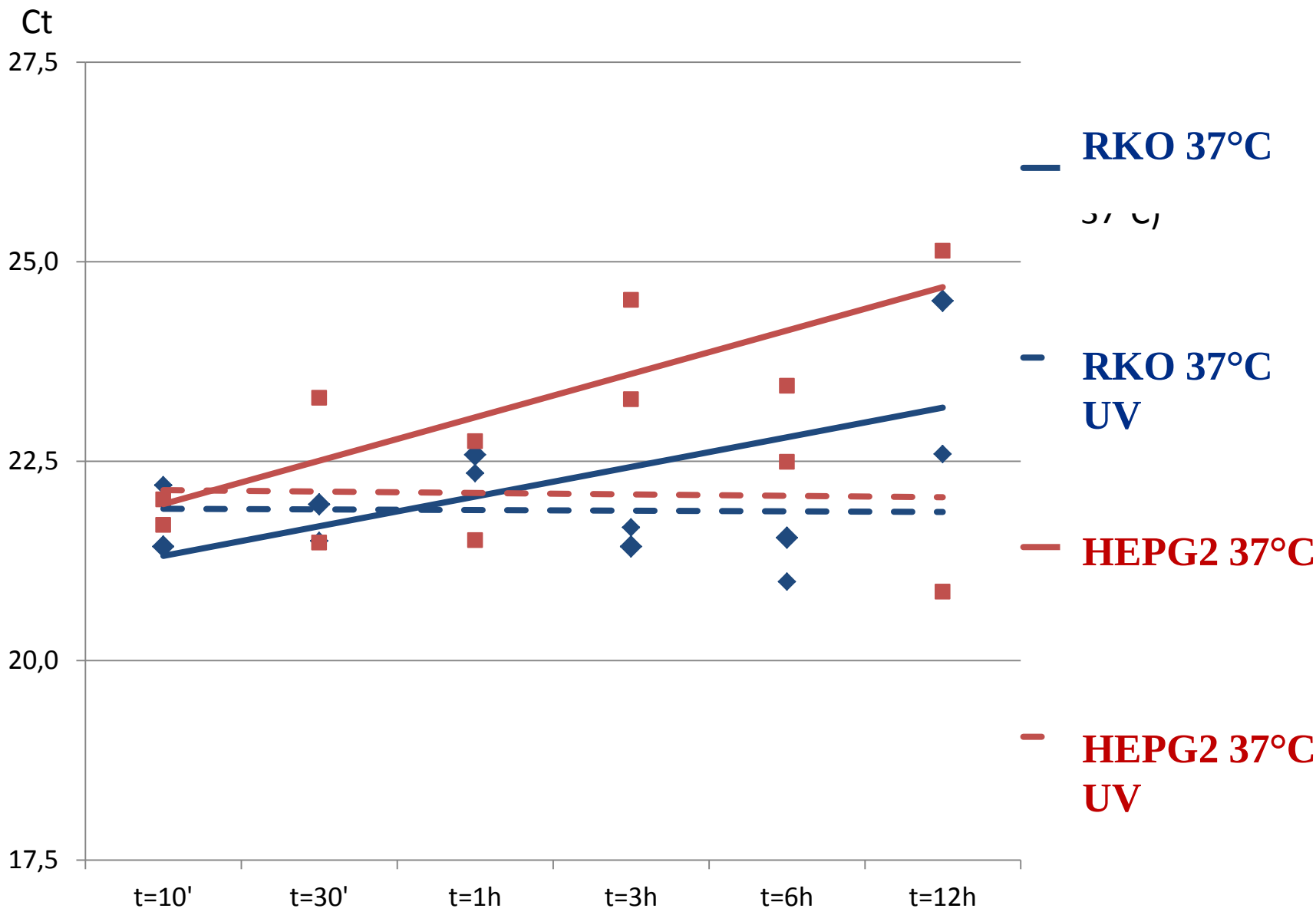
installed in “dirty room“ adjacent to surgery suite.

Elimination of formalin in surgery theatre.

Transport biospecimens in “as fresh conditions” to the

Pathology lab.

C-FOS mRNA at 37°C vs 37°C under vacuum



STANDARDIZATION OF FIXATION TIME

1-Formalin time fixation/saline cycle substitution can be settled.

2-Formalin is drained after fixation time has expired.

3-Saline solution is pumped in to stop fixation, and to preserve the specimen.

FixMATE

OPEN ACCESS Freely available online

PLoS one

Formalin Fixation at Low Temperature Better Preserves Nucleic Acid Integrity

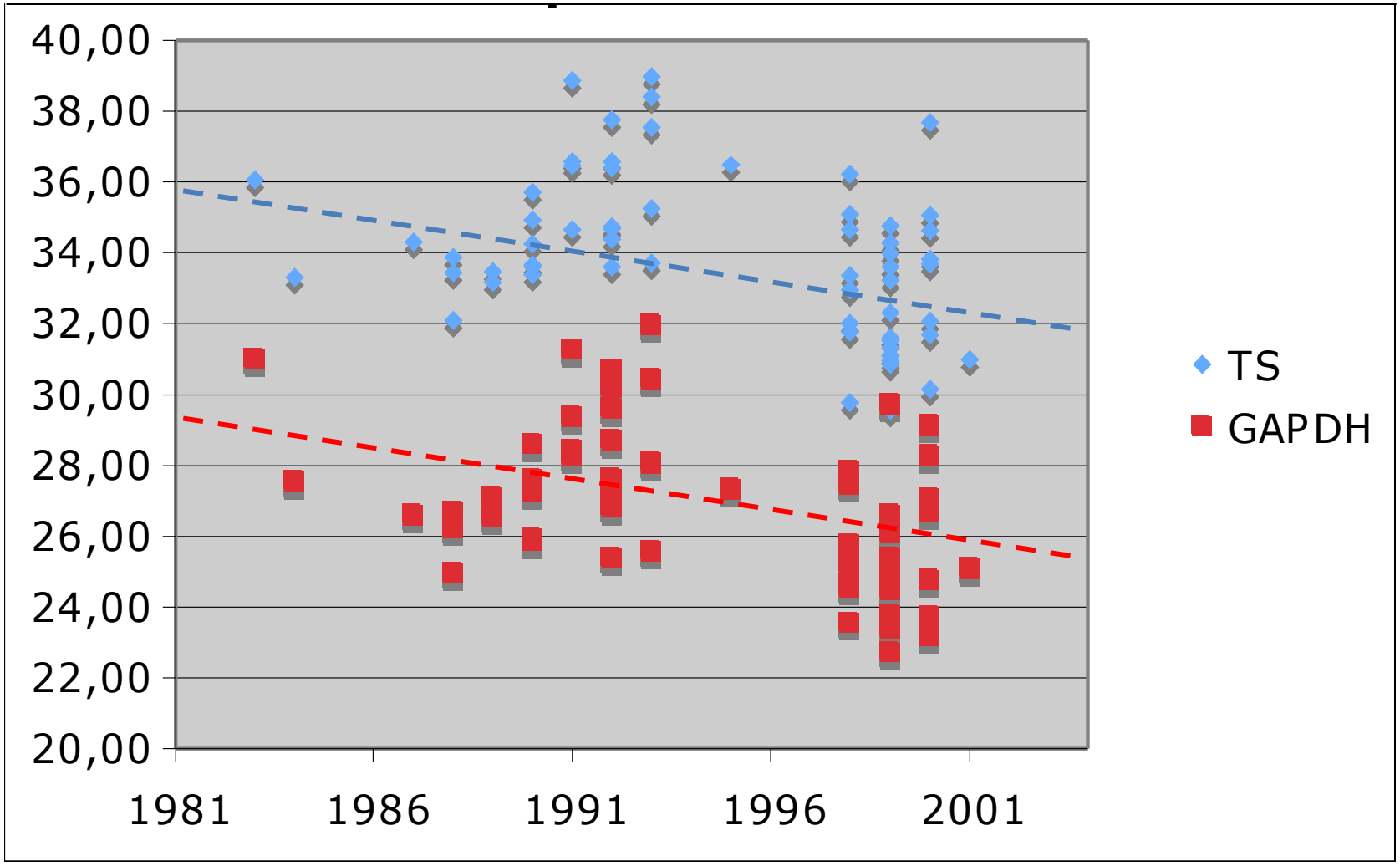
Gianni Bussolati[1], Laura Annaratone[1], Enzo Medico[2]*, Giuseppe D'Armento[1], Anna Sapino[1]*

1 Department of Biomedical Sciences and Human Oncology, University of Turin, Turin, Italy, 2 Department of Oncological Sciences, Institute for Cancer Research and Treatment (IRCC), University of Turin, Candiolo, Italy

Abstract

Fixation with formalin, a widely adopted procedure to preserve tissue samples, leads to extensive degradation of nucleic acids and thereby compromises procedures like microarray-based gene expression profiling. We hypothesized that RNA fragmentation is caused by activation of RNAses during the interval between formalin penetration and tissue fixation. To prevent RNAse activation, a series of tissue samples were kept under-vacuum at 4°C until fixation and then fixed at 4°C, for 24 hours, in formalin followed by 4 hours in ethanol 95%. This cold-fixation (CF) procedure preserved DNA and RNA, so that RNA segments up to 660 bp were efficiently amplified. Histological and immunohistochemical features were fully comparable with those of standard fixation. Microarray-based gene expression profiles were comparable with those obtained on matched frozen samples for probes hybridizing within 700 bases from the reverse transcription start site. In conclusion, CF preserves tissues and nucleic acids, enabling reliable gene expression profiling of fixed tissues.

Real-time PCR: Ct comparison of TS and GAPDH by year of fixation



SOURCES OF CLINICAL RESEARCH AND DIAGNOSTICS VARIABILITY

#Tissue and macromolecule pre-analytical preservation

#Heterogeneity at the clinical, morphological or molecular level

#Selection and standardization of analytical procedures

TISSUE HETEROGENEITY

CLINICAL HETEROGENEITY*: related to different patient conditions (different tumor type, age, therapy, etc.)*

TISSUE RELATED HETEROGENEITY*:*

*-**Related to tissue complexity** (fibrosis, flogosis, necrosis, normal residual tissues…)*

*-**Related to histological heterogeneity** (different histological pattern of the same tumor)*

MOLECULAR HETEROGENEITY:

-Genetic clonal evolution (MSI, CI,…)

-Epigenetic clonal evolution (CIMP,…)

-Phenotypic plasticity (cancer stem cells, hypoxia, others…)

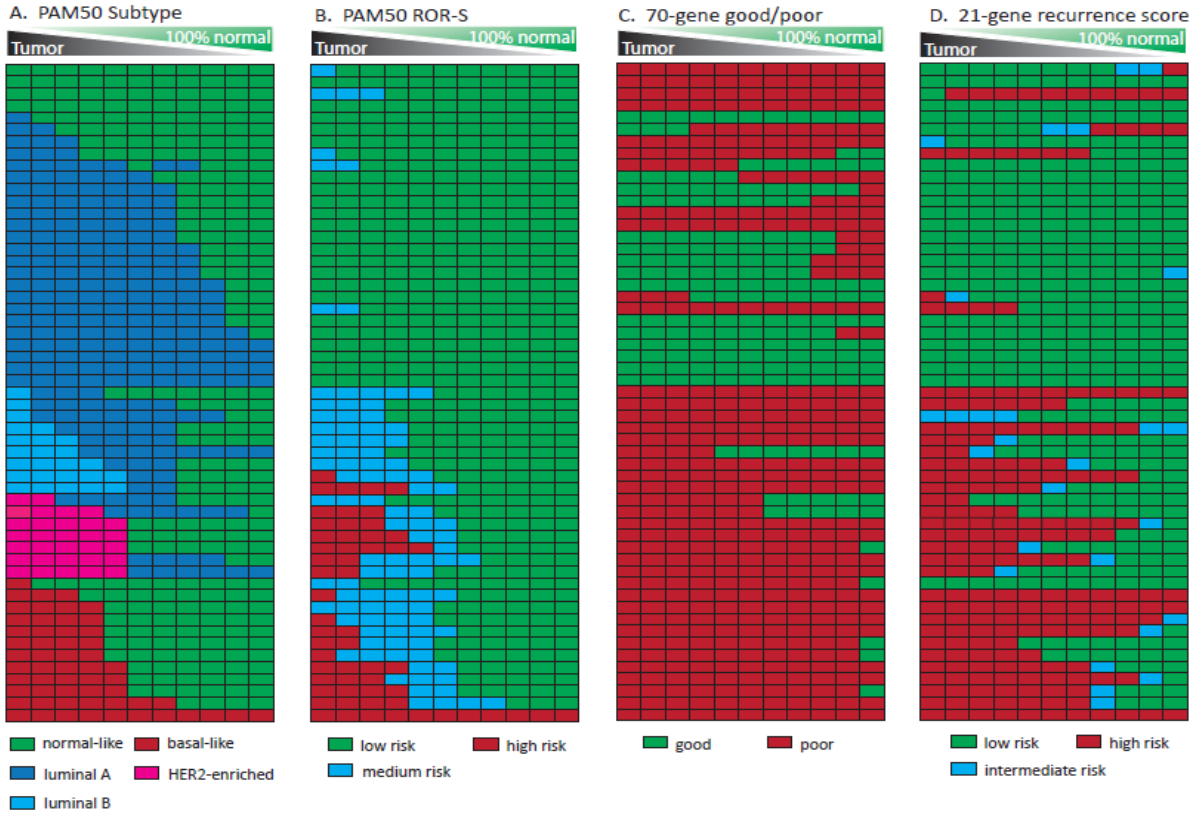
Elloumi *et al. BMC Medical Genomics* 2011, **4**:54
http://www.biomedcentral.com/1755-8794/4/54

BMC Medical Genomics

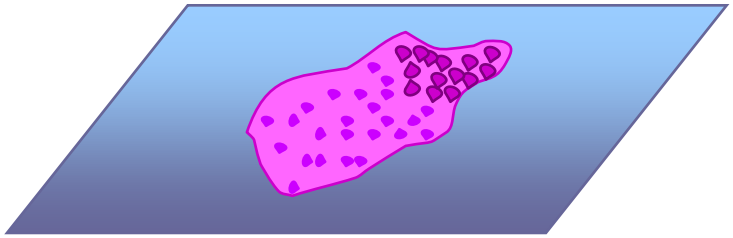
RESEARCH ARTICLE Open Access

Systematic Bias in Genomic Classification Due to Contaminating Non-neoplastic Tissue in Breast Tumor Samples

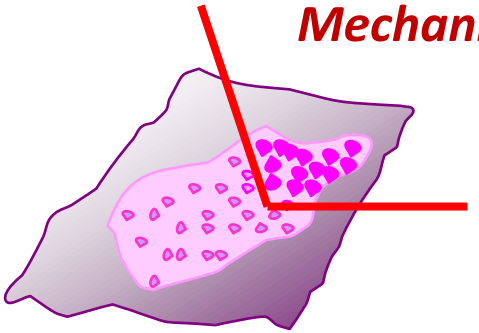
Fathi Elloumi[1], Zhiyuan Hu[1], Yan Li[1], Joel S Parker[2], Margaret L Gulley[1,3], Keith D Amos[1,4] and Melissa A Troester[1,5*]



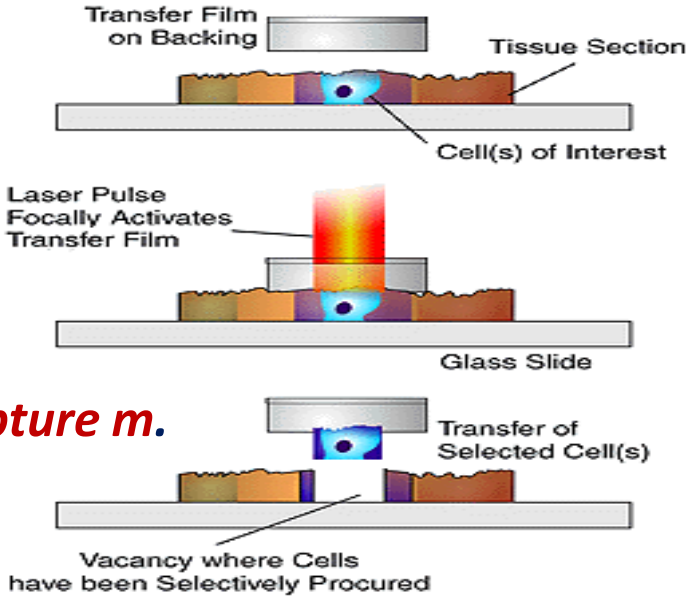
Tissue selection by micro-dissection



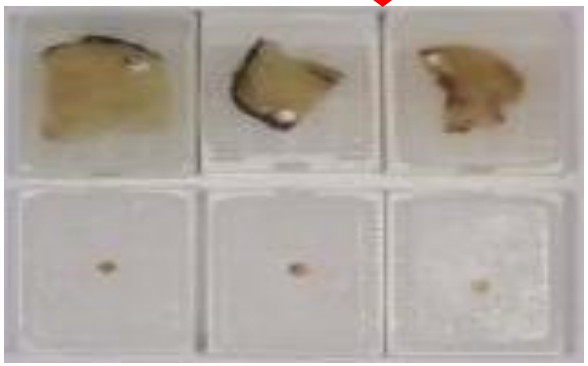
Mechanical m.



Laser capture m.



Tissue arrayer m.



PRACTICAL APPROACH TO TACKLE PRECLINICAL T. HETEROGENEITY

SMALL BIOPSY

1-Histological evaluation of tissues

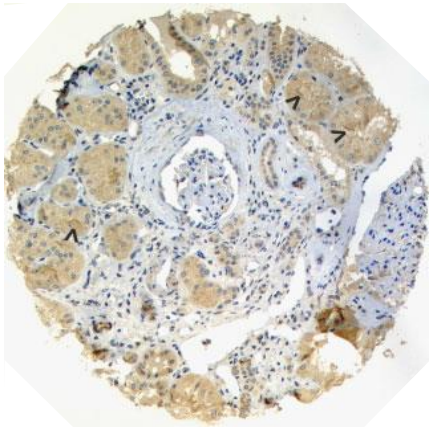
2-Possible micro-dissection (stroma, normal t. residues,…)

3-Digital record of the selected tissues

SURGICAL SPECIMEN

1-Histological evaluation of tissues with topographical definition (identification of the infiltrative border of the t.)

2-Micro-dissection: single or multiple sampling, depending on the type of lesion and on the histological pattern:
a-in single sample, this should be taken from the infiltrative border, with the minimal stromal component
b-for multiple sampling the topographical location of the micro-dissected areas should be recorded.



3-Digital record of the micro-dissected areas as integral part of result evaluation (specific IHC could help a morphometric cancer cells and stroma evaluation)

4-Very short time between micro-dissected area sections cutting and the extraction of nucleic acids (especially RNA), otherwise this could be another preanalitycal source of variability

TMA ARRAYER MICRODISSECTION

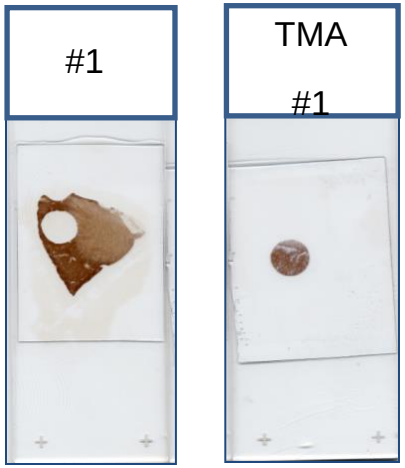
Core diametre (mm)	Core surface (mm2)	Sections for 1 cm^2
3	7.065	14
5	19.62	5

GENE EXPRESSION QUANTITATIVE ANALYSIS - Ct

Gene	β-Actin mRNA		CDK2 mRNA	
Sample	Coring 1	Coring 2	Coring 1	Coring 2
1	21.48	21.64	29.43	29.16
2	28.45	28.22	32.92	32.92
3	23.71	23.72	32.32	31.99
4	28.84	28.75	33.29	33.29
5	28.08	28.36	33.24	33.24

#Treatment after coring 50°C for 30 min plus 60°C for 10 min (especially for 5mm cores)
#Expected RNA yield from 5 sections (1cm^2), 5 µm thick: 5 - 25 µg (related to tissue type and extraction method)

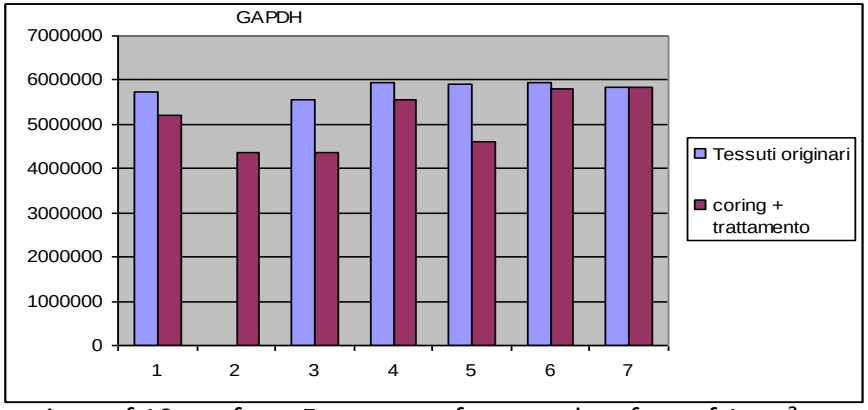
The previously reported approach should be fitted to specific type of analysis and corrected by direct clinical performance experience:

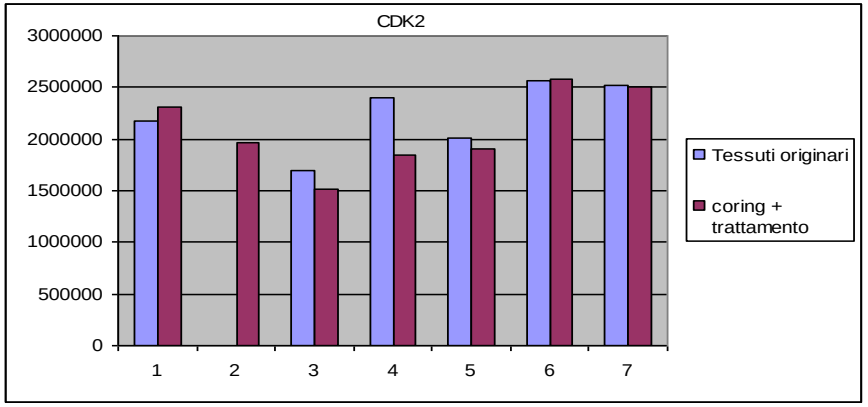


**Cts after real time amplification of 10 ng of cDNA after reverse transcription with random hexamers - not standardized Cts*

Gene	β-Actin mRNA		CDK2 mRNA	
Sample	Tissues	Coring	Tissues	Coring
1	23.01*	21.64	30.11	29.16
2	28.48	28.22	33.13	32.92
3	24.53	23.72	31.76	31.99
4	29.72	28.75	33.25	33.29
5	29.15	28.36	33.56	33.24

QUANTITY PROTEIN EXTRACTION





-5 sections of 10 µm from 5mm cores for a total surface of 1 cm^2, compared with a similar surface of the original tissue. -Extraction by Qproteome FFPE Tissue Kit.. Total protein concentration by NanoPhotometer™.

- DotBlot: 10 µl of1:200 of protein solution spotted on membrane. Antibodies against GAPDH and CDK2. Developed by ECL on Immobilon membrane. Analysis of the dots by Versadoc with ImageJ software .

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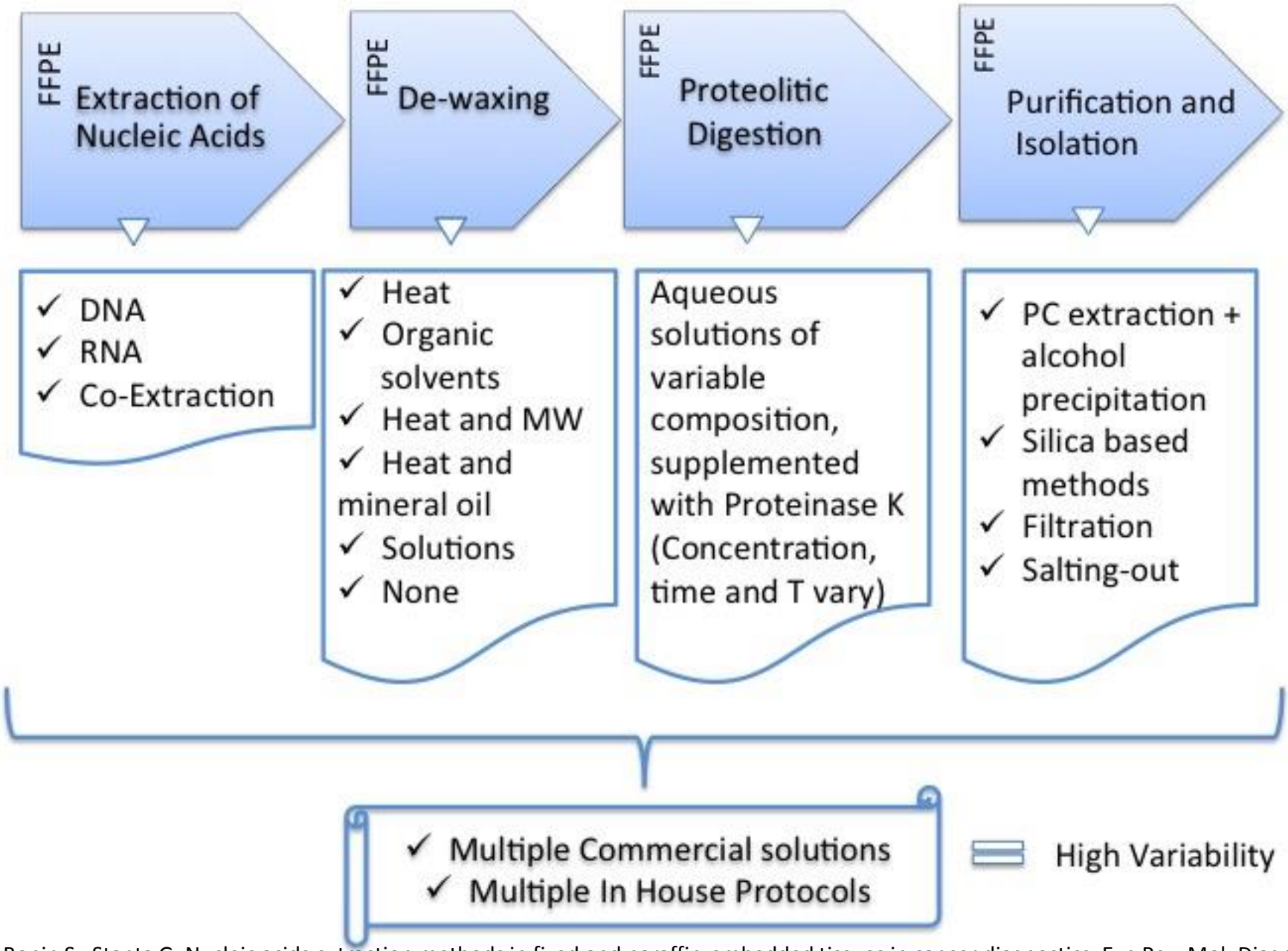


Bonin S , Stanta G. Nucleic acids extraction methods in fixed and paraffin-embedded tissues in cancer diagnostics. Exp Rev Mol. Diagn. 2013,13.

DNA AND RNA EXTRACTION METHODS REPORTED IN LITERATURE

First Author [Ref]	DNA Methods, kit and Manufacturer	Note
Bonin [24]	QIAamp DNA Mini Kit (Qiagen), in house methods (PCI*), NucleoSpin Tissue Kit (Macherey Nagel), DNeasy FFPE kit (Qiagen).	
Dedhia [50]	QIAamp DNA Mini Kit (Qiagen), PCI, in house method	
Funabashi [28]	PCI, in house method QIAmp Mini Kit (Qiagen)	
Huijsmans [23]	Heat treatment, QIAmp DNA blood –mini-kit (Qiagen), EasyMAG (NucliSens), Gentra-capture-column-kit (Gentra Systems)	
Kotorashvili [31]	Trizol (Invitrogen), All Prep DNA/RNA FFPE kit (Qiagen), RecoverAll (Ambion), Qiamp DNA FFPE kit (Qiagen)	Co-extraction of RNA and DNA
Ludyga [29]	QIAmp DNA FFPE kit (Qiagen), in house method (PCI), FFPE RNA/DNA purification kit (Norgen)	Long term preserved tissues
Munoz-Cadavid [30]	QIAamp DNA FFPE Tissue Kit (Qiagen), TaKaRa Dexpat (Takara), PureLink Genomic DNA Mini Kit (Invitrogen), WaxFree DNA (TrimGen), QuickExtract FFPE DNA Extraction Kit (Epicenter Biotechnologies)	For detection of fungal DNA
Okello [20]	Mammalian Genomic DNA Miniprep Kit (Sigma), Tri-reagent solution (Ambion), RecoverAll (Ambion), in house method (PCI)	Archival autopsy tissues
Torrente [85]	In house method (PCI), QIAmp DNA FFPE kit (Qiagen)	
Turashvili [10]	QIAmp DNA FFPE (Qiagen), RecoverAll (Ambion), Trimgen wax free DNA (Trimgen), in house method (PCI)	

First Author [Ref]	RNA Methods, kit and Manufacturer	Note
Abramoviz [39]	RecoverAll (Ambion), High Pure paraffin kit (Roche), RNeasy FFPE (Qiagen), ArrayGrade FFPE RNA isolation (SuperArray)	Application to DASL** assay
Bonin [24]	RNeasy FFPE kit (Qiagen), High Pure FFPE RNA (Roche), in house methods (PCI* and Trizol purification)	
Doleshal [35]	RNeasy FFPE kit (Qiagen), Absolutely RNA FFPE kit (Stratagene), High Pure FFPE RNA Micro (Roche), PureLink RNA Isolation kit (Invitrogen), RecoverAll (Ambion)	For Micro RNA expression analyses
Jacobson [86]	RNeasy FFPE kit (Qiagen), High Pure FFPE RNA (Roche), RecoverAll (Ambion)	Application to Microarray
Kotorashvili [31]	Trizol (Invitrogen), All Prep DNA/RNA FFPE kit (Qiagen), RecoverAll (Ambion)	Co-extraction of RNA and DNA
Linton [87]	Optimum FFPE RNA isolation (Ambion), RNeasy FFPE kit (Qiagen),	Optimised
Ludyga [29]	RNeasy FFPE kit (Qiagen), in house method (PCI); FFPE RNA/DNA purification kit (Norgen)	Long term preserved tissues
Okello [20]	Absolutely RNA FFPE Kit (Stratagene), Tri-reagent solution (Ambion), High Pure FFPE RNA (Roche), High Pure FFPE RNA Micro Kit (Roche), in hose method (PCI), Trimgen Waxfree RNA kit (Trimgen), RecoverAll (Ambion)	Archival autopsy tissues
Ribeiro-Silva [38]	RecoverAll (Ambion), High Pure FFPE RNA (Roche), Absolutely RNA FFPE kit (Stratagene), FormaPure kit (Agencourt)	
Roberts [16]	RecoverAll (Ambion), Paradise Whole Transcripl Rt reagent system (Arcturus Bioscience), High Pure FFPE RNA (Roche), Purelink FFPE RNA (Invitrogen), FormaPure RNA (Agencourt Bioscience Corporation)	
Ton [40]	RNeasy FFPE kit (Qiagen), High Pure FFPE RNA (Roche),	Application to DASL** assay
Turashvili [10]	RNeasy FFPE kit (Qiagen), Trimgen Wax free RNA (Trimgen), RecoverAll (Ambion), in house method (PCI)	

PCI Phenol Chloroform Isolation - DASL Assay: cDNA-mediated Annealing, Selection, Extension, and Ligation Assay

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

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**.

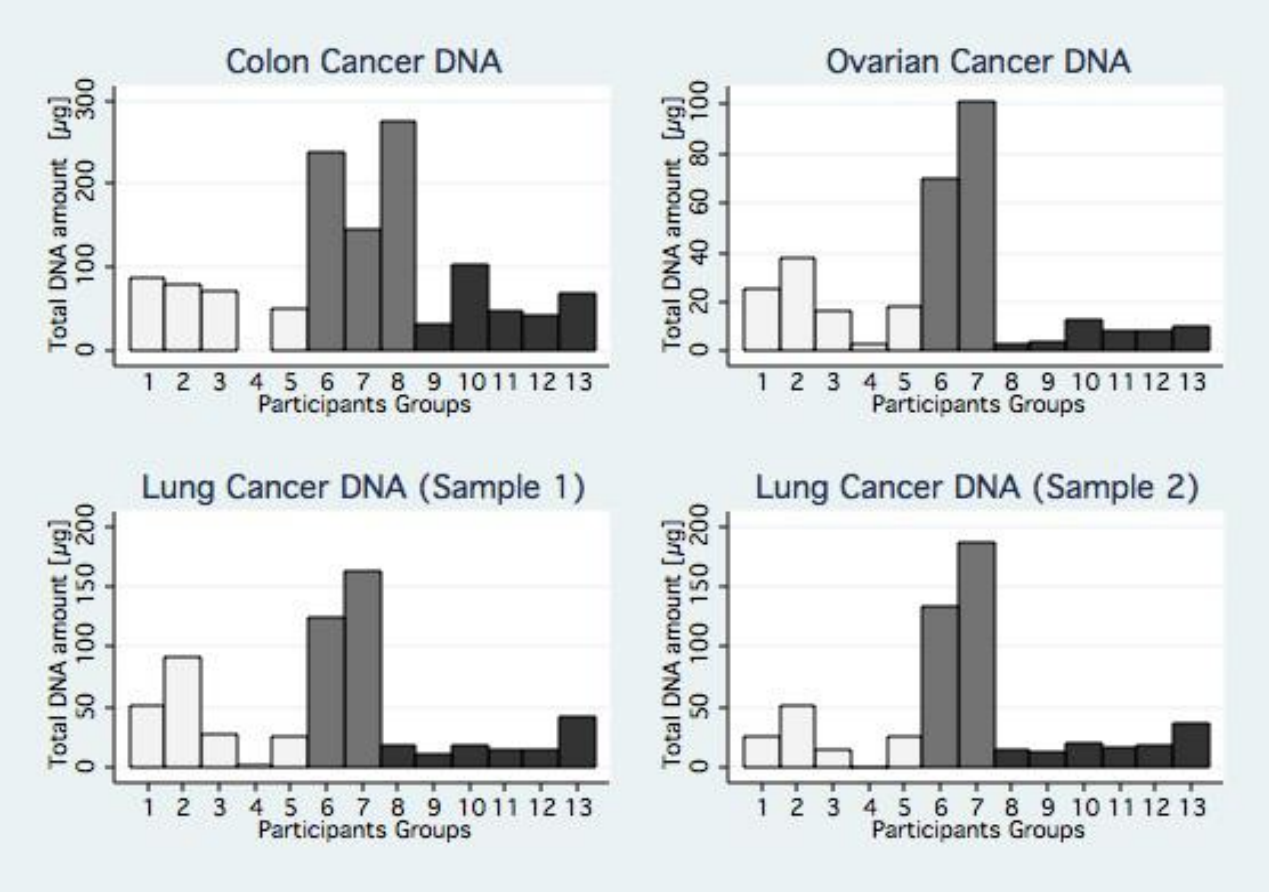
Earlier this year, my colleagues and I experienced every scientist's worst nightmare. Twelve months of experiments were deemed useless after we showed that a recommended negative control for chemically synthesized stretches of RNA (microRNA mimics), bought from a biotechnology company, was inappropriate. 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 ▶

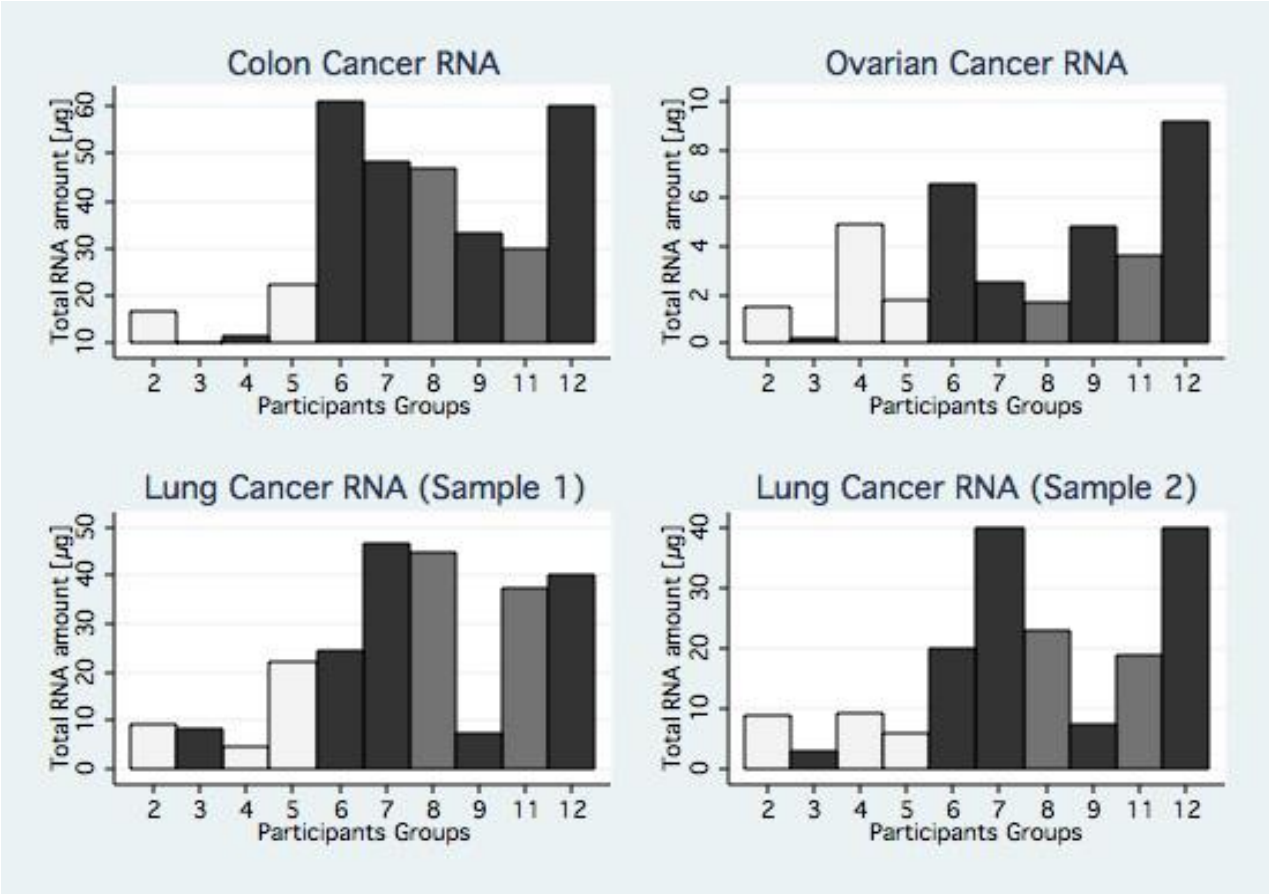
DNA EXTRACTION VARIABILITY



1-5: purification with phenol/chloroform and precipitation or monophasic solution
6-8: crude extracts – with protein debris and over-estimation of DNA amount
9-13: commercial kits with absorption on silica columns

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 in print

RNA EXTRACTION VARIABILITY



***Light grey bars* -RNA extraction with home- made protocols**
***Medium grey bars* -RNA extraction with mono-phasic commercial solutions**
***Dark grey bars* -RNA extraction with commercial kits based on the use of silica based columns**

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" Virchows Arch. 2010 Sep;457(3):309-17

CONCENTRATION OF NUCLEIC ACIDS

#SPECTROPHOTOMETRIC METHOD FOR DNA AND RNA

For ds DNA $[DNA] = A_{260} * \text{dilution factor} * 50 * 10^{-3}$ (µg/µl)

For ss DNA $[DNA] = A_{260} * \text{dilution factor} * 33 * 10^{-3}$ (µg/µl)

For RNA $[RNA] = A_{260} * \text{dilution factor} * 40 * 10^{-3}$ (µg/µl)

The ratio A_{260} / A_{280} is used to evaluate the purity level of nucleic acids with respect to proteins, phenol, etc. Contamination by other nucleic acids is not considered. A ratio higher than 1.5 and ≤2 means good purity.

The ratio A_{260} / A_{230} is used to evaluate the contamination level by carbohydrates and salts. A ratio

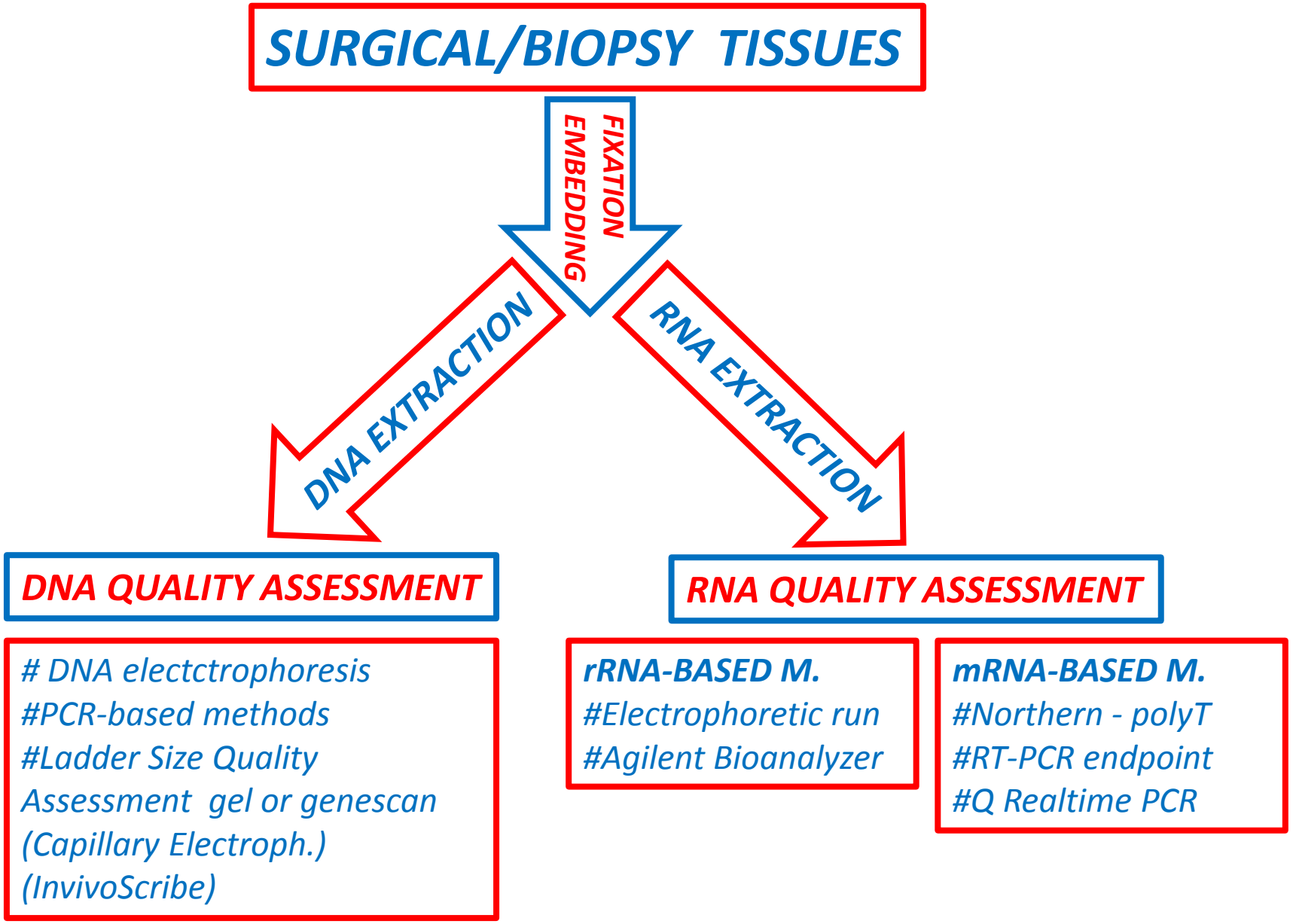
higher than 1.5 means that A_{260} reflects the real concentration of the nucleic acid.

#FLUOROMETRIC METHOD FOR DNA OR RNA

Incubation with fluorochrome (Hoechst 33258 or PicoGreen for DNA and RiboGreen for RNA) and comparison with standard curve. Fluorimeter based.

#ETHIDIUM BROMIDE METHODS

Agarose plate or minigel (comparison with known standards). Spotting directly the tested DNA on agarose gel prepared in a small Petri capsule together with 4 or 5 reference DNAs, of known concentration. Evaluation at a UV transilluminator by direct comparison.


SURGICAL/BIOPSY TISSUES
FIXATION
EMBEDDING
DNA EXTRACTION
RNA EXTRACTION
DNA QUALITY ASSESSMENT
DNA electctrophoresis
#PCR-based methods
#Ladder Size Quality Assessment gel or genescan (Capillary Electroph.)
(InvivoScribe)
RNA QUALITY ASSESSMENT
rRNA-BASED M.
#Electrophoretic run
#Agilent Bioanalyzer
mRNA-BASED M.
#Northern - polyT
#RT-PCR endpoint
#Q Realtime PCR

SURGICAL/ BIOPSY TISSUES

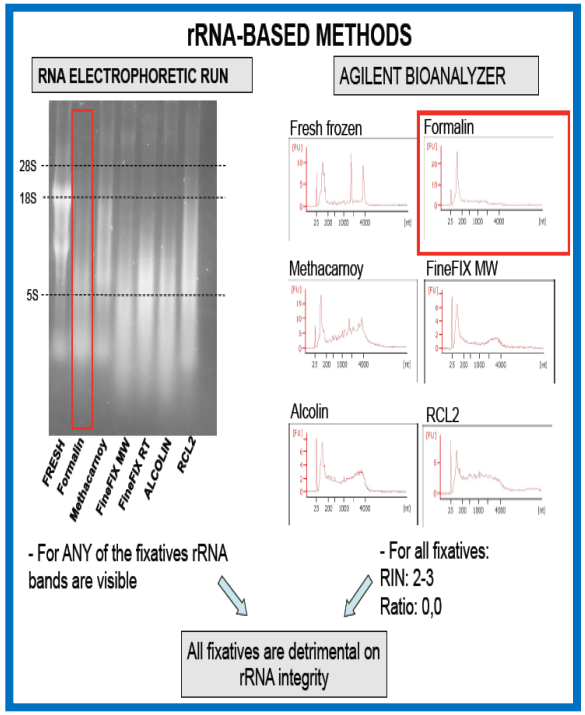
FIXATION EMBEDDING

RNA EXTRACTION

RNA QUALITY ASSESSMENT

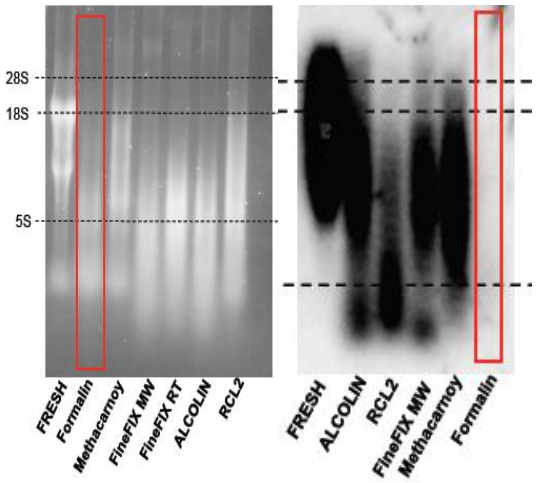
rRNA-BASED M.
#Electrophoretic run
#Agilent Bioanalyzer

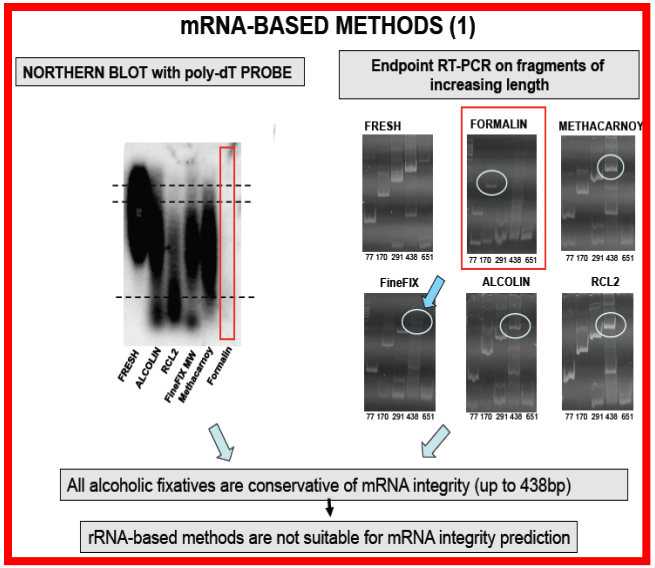
mRNA-BASED M.
#Northern - polyT
#RT-PCR endpoint
#Q Realtime PCR

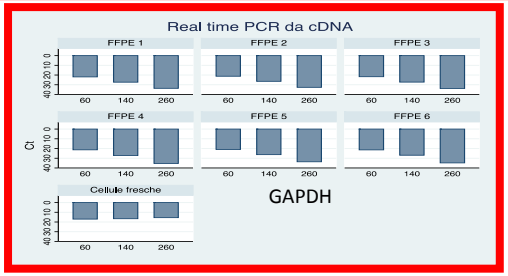


rRNA Gel Electro-phoresis

mRNA Northern B. PolyT







Isabella Dotti.; Serena Bonin.; Giorgio Basili; Ermanno Nardon; Alessandro Balani;; Salvatore Siracusano; Fabrizio Zanconati; Silvia Palmisano; Nicolò De Manzini; Giorgio Stanta, "Effects of formalin, methacarn and FineFIX fixatives on RNA preservation" Diagn Mol Path 2009

DNA QUALITY STANDARDIZATION

#The quantity of extracted DNA varies with the method used, but very often the quantity is not related to the quality as maximum amplifiable length.

#The purity of the extraction measured by spectrophotometric methods is not related to the quality as maximum amplifiable length.

#If we need good quality DNA, extraction protocols with purification steps should be chosen and the best method could vary depending on the type of analysis performed.

#We need to standardize the "quality evaluation test" and the cut-off is related to the type of analysis. On this test we should base the inclusion of the sample in the analysis (repeated extraction or inadequate sample).

#Quantity of DNA is also important because it can allow the detection of the searched alteration or not in a situation of heterogeneity.

RNA QUALITY STANDARDIZATION

#The quantity of extracted RNA varies with the method used, usually many commercial kits give maximum quantity.

#The purity of the extraction measured by spectrophotometric methods is not related to the quality as maximum amplifiable length.

#The use of some commercial kits also gives the best quality of extracted RNA, but the use of a specific extraction kit is not sufficient to standardize the extracted quality as high level of amplifibility (RNA quantity and quality vary depending on the lab performance and on the SOPs).

#We need to standardize the "quality evaluation test" , but we cannot use rRNA based test. On this test we should base the inclusion of the sample in the analysis (repeated extraction or inadequate sample).

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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,*]

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[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."

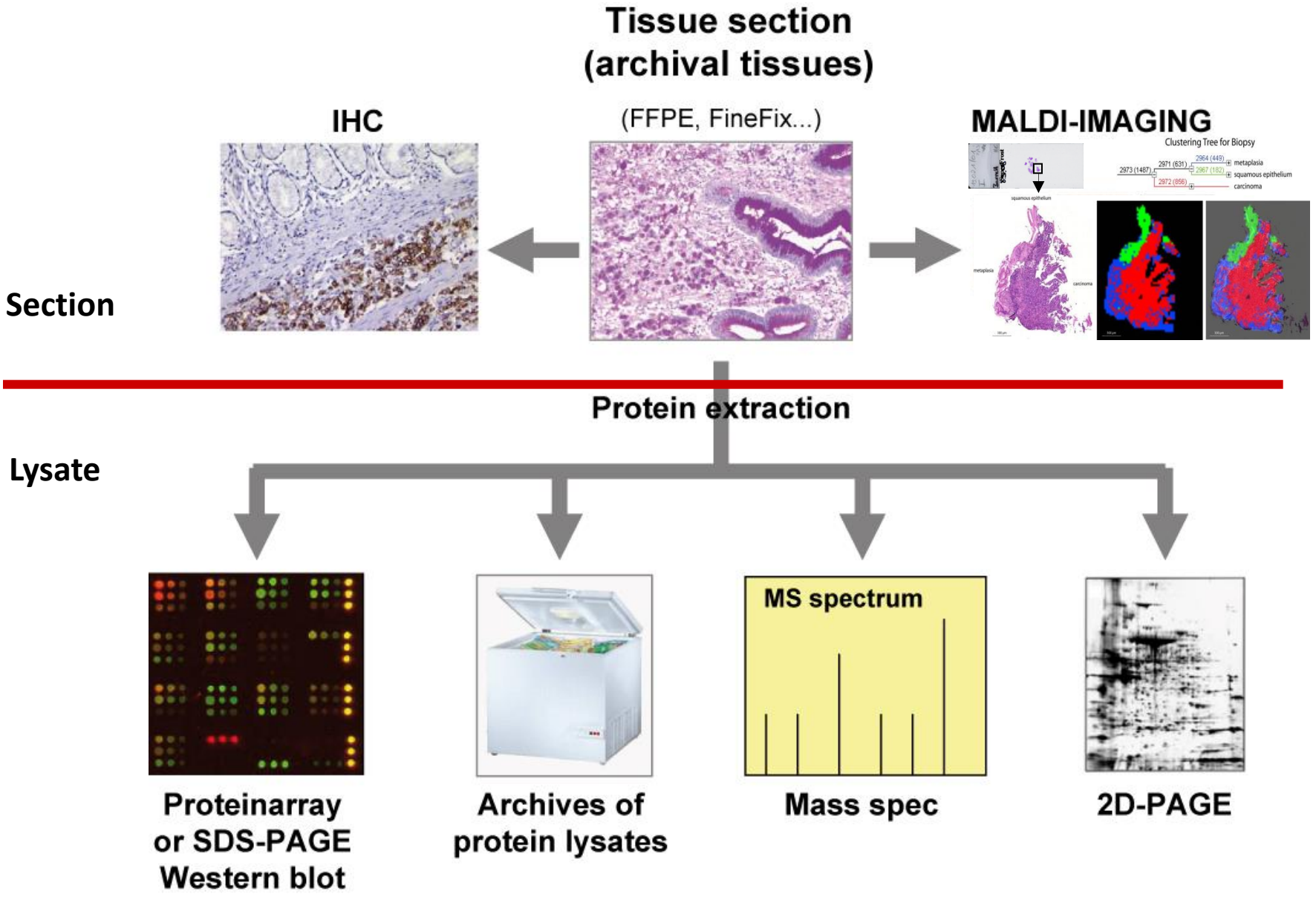
Table 4
Summary of case study results.

	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.

Proteomics in archive tissues

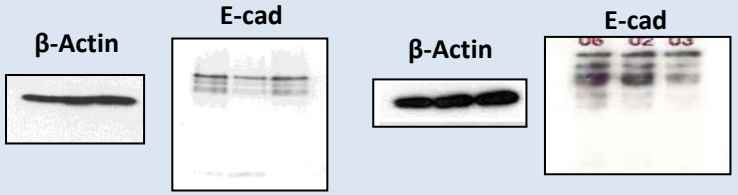


KF Becker

Protein extraction from FFPE samples using Qproteome FFPE kit

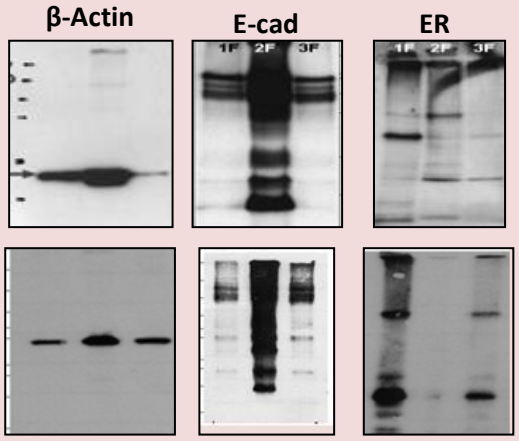
Western blot analyses

Lung cancer



Munich Trieste

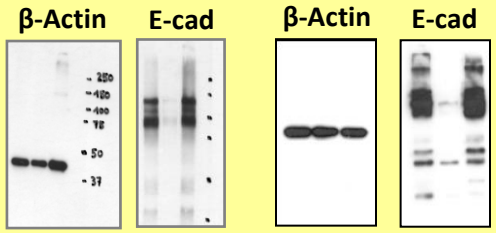
Breast cancer



Munich

Trieste

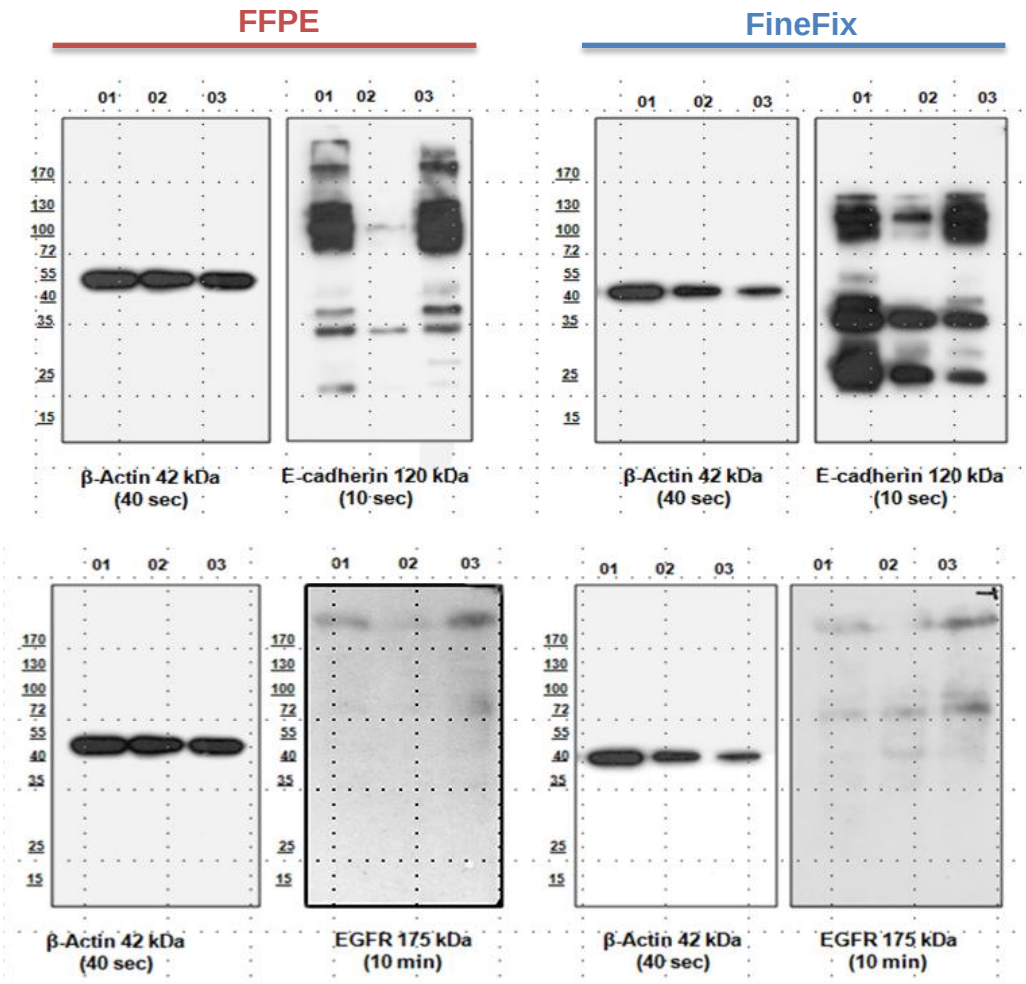
Colon cancer



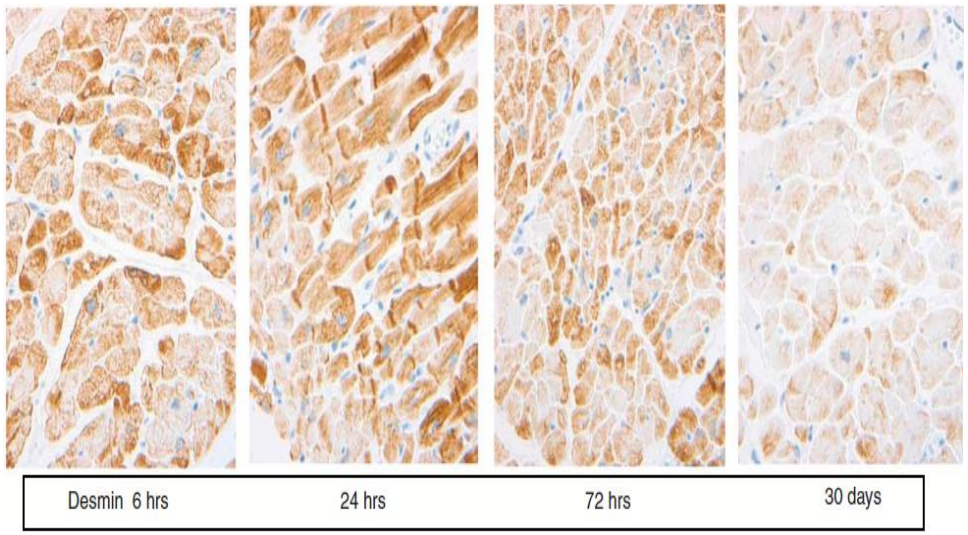
Munich Trieste

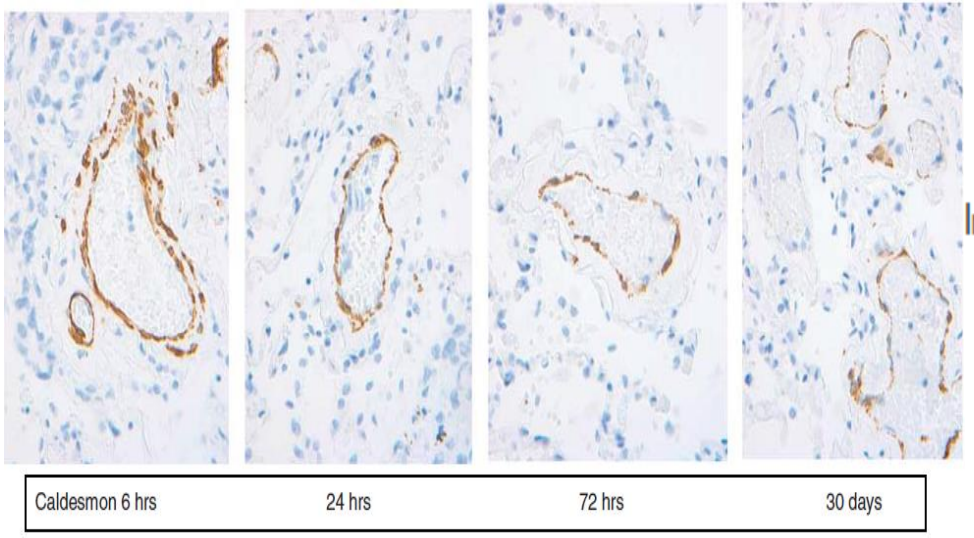
Protein extraction from matched FFPE and FineFix colon cancer samples using Qproteome FFPE kit

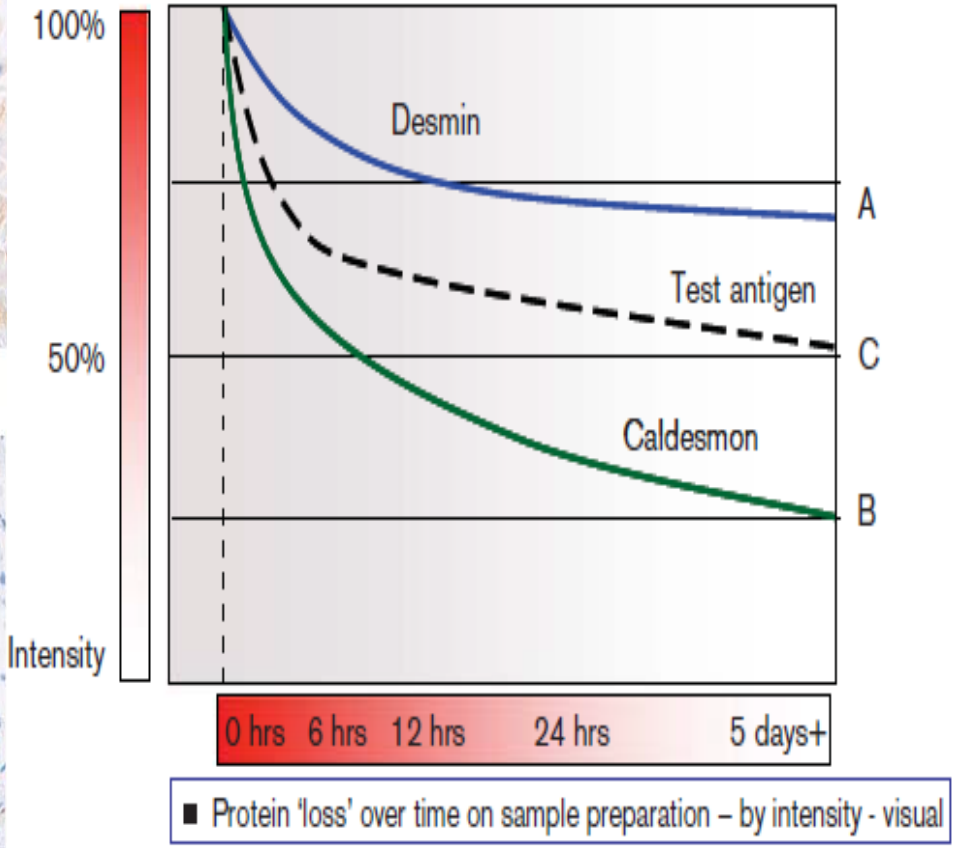
Western blot analyses



Proteins degradation references in FFPE tissues



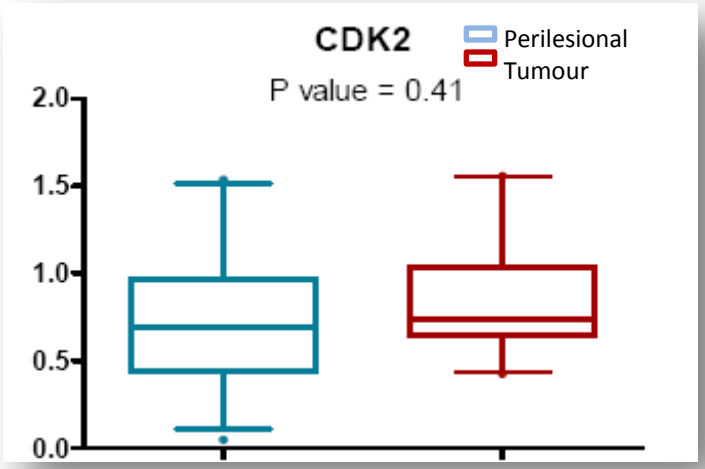


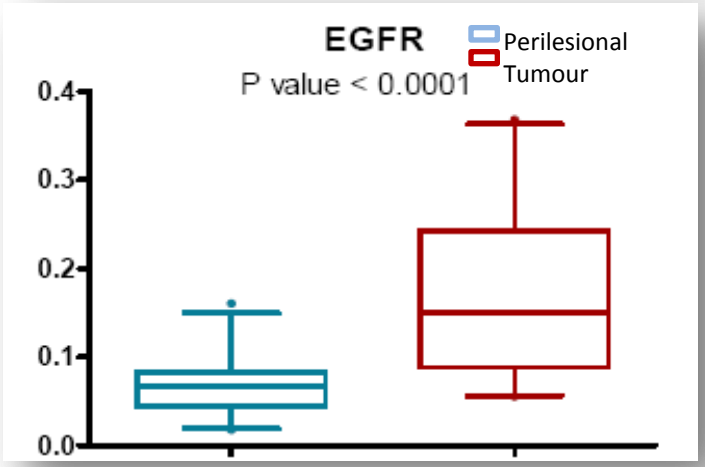


Karl-Friedrich Becker, Clive R. Taylor, Appl Immunohistochem Mol Morphol 2011;19:1–9

mRNA versus protein analysis

mRNA





PROTEIN