

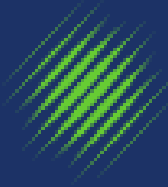


- EU SPIDIA Project -

Pre-analytical handling of biosamples; optimising biobank sample quality for protein and nucleic acid studies

Symposium on Biosample Quality
Guy's Hospital, London, May 9th 2012

Dr. Daniel Grölz
SPIDIA (QIAGEN)



Standardization and Improvement of Generic Pre-analytical Tools and Procedures for *In Vitro* Diagnostics

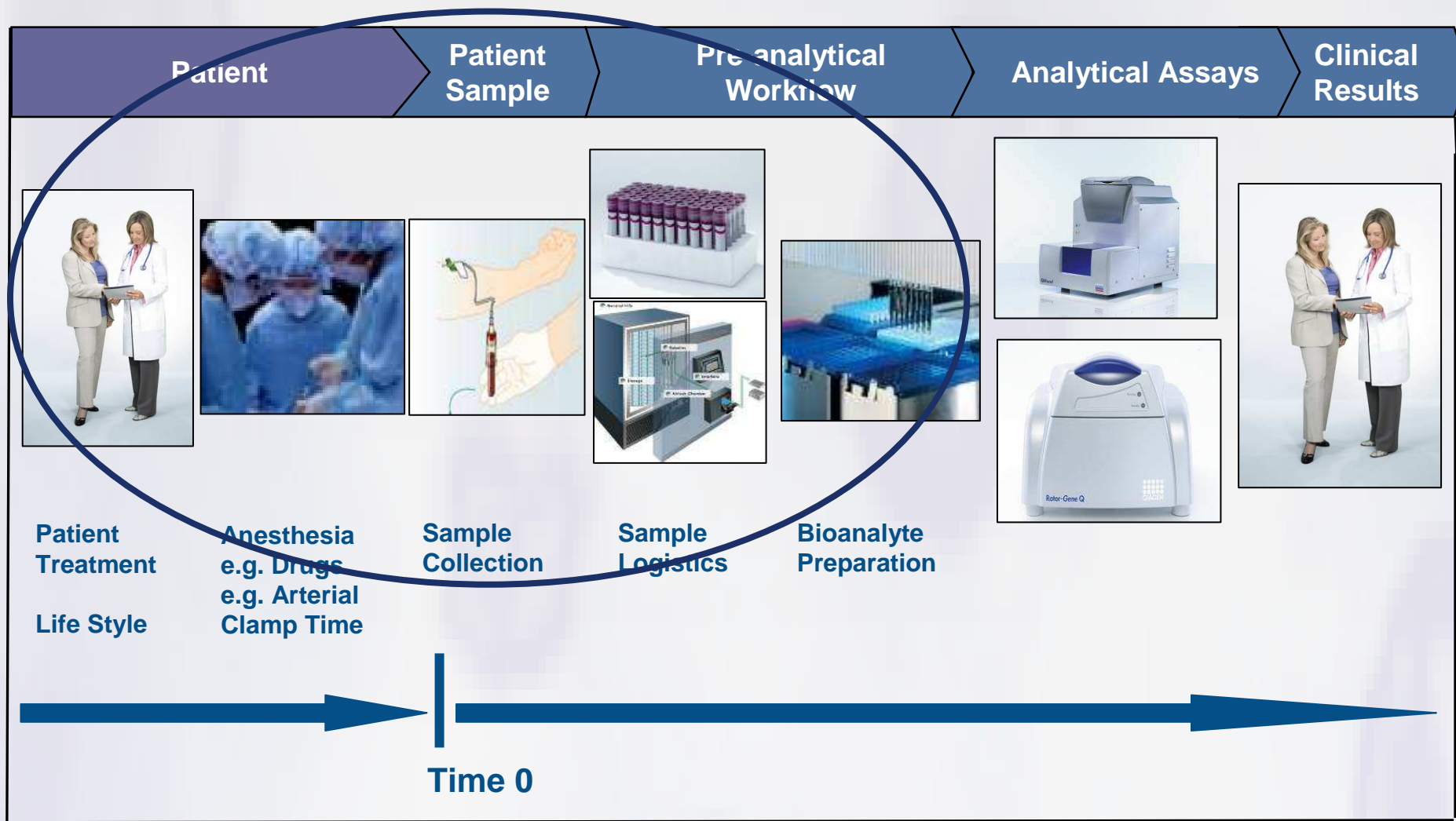
■ **SPIDIA**

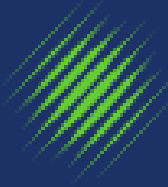
- **Project Facts & Goals**
- **New Technologies & Tools**
- **Guidelines & Dissemination**
- **Evaluation of PAXgene Tissue**

SPIDIA

Molecular Diagnostic Workflow

From Patients to Clinical Results

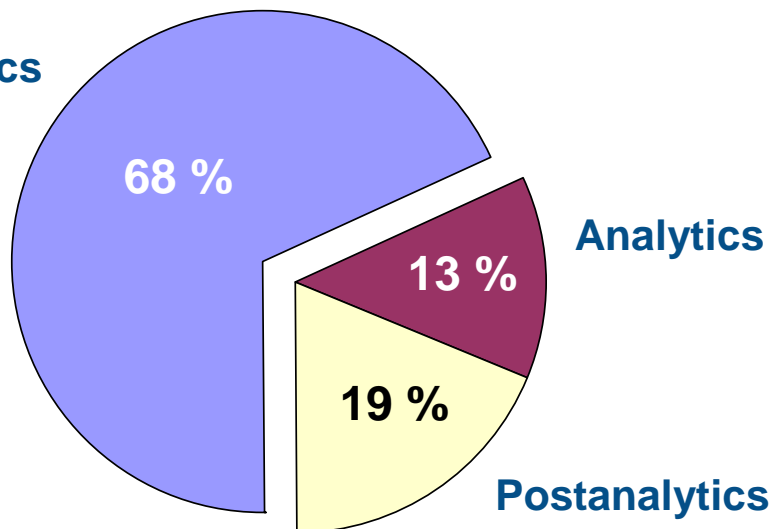




“Preanalytical errors still account for nearly 60%-70% of all problems occurring in laboratory diagnostics, most of them attributable to mishandling procedures during collection, handling, preparing or storing the specimens”.

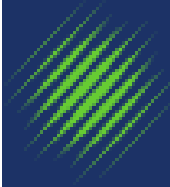
Lippi G. *et al.*. Preanalytical quality improvement: from dream to reality. Clin Chem Lab Med. 2011 Jul; 49(7):1113-26. Epub 2011 Apr 25.

Preanalytics



Costs of ~ 460,000 \$ / year in an average German hospital caused by pre-analytical errors

Frost & Sullivan 2011 on behalf of BD

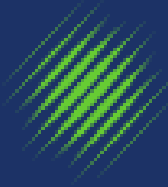


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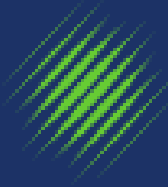
Improvements Needed



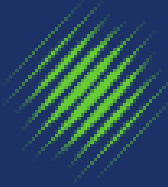
- Understanding biomolecule profile changes during pre-analytical workflows
- New pre-analytical technologies preventing biomolecule profile changes
- New evidence based standards and guidelines
- Detailed description of biological / clinical samples histories for research, biobanking, diagnostics
- Ideally – sample quality markers



- New pre-analytical tools & technologies (Blood, Plasma, Tissue, Swabs)
- Sample quality markers (Blood, Tissue)
- Pan-European guidelines for preanalytics (Blood, Tissue)
- Training and dissemination



- Program European Commission FP7-HEALTH
- Consortium 7 public research organizations
8 companies
1 standards organization (CEN)
- Coordinator QIAGEN GmbH
- Run Time October 2008 – September 2012
(prolongation requested)
- Budget 13 Mio € (9 Mio € EC contribution)
- Co-operations NCI / OBBR, CLSI, EFCC, BBMRI and other
international initiatives and organizations
- Web page www.spidia.eu
- Newsletter



Standardization and Improvement of Generic Pre-analytical Tools and Procedures for *In Vitro* Diagnostics

■ **SPIDIA**

- Project Facts & Goals
- **New Technologies & Tools**
- Pan-European Guidelines & Dissemination
- Evaluation of PAXgene Tissue



New Technology & Tools Ongoing Developments

■ Tissue

- Stabilization of morphology, antigenicity, DNA, RNA, proteome

■ Fine Needle Aspirates

- Stabilization of morphology, antigenicity, DNA, RNA, proteome

■ Plasma

- New stabilization technologies

■ Whole Blood

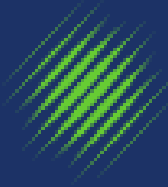
- New stabilization technologies

■ Swabs

- Stabilization and improved processing for molecular analysis

■ Stabilized Whole Blood

- Integrated automated sample-to-result workflows (cellular RNA, ncRNAs incl. miRNAs)

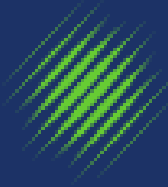


■ **Quality marker for blood and tissue**

- To monitor changes in clinical sample materials
- Ischemia time, storage time and temperature

■ **Quality markers blood measuring RNA up- & down-regulation**

- >180 micro arrays (time course experiments)
- 17 marker candidates (specific RNA degradation or gene down regulation, specific RNA gene induction)
- Technical assay validation
- Next step: Performance validation within larger donor cohorts



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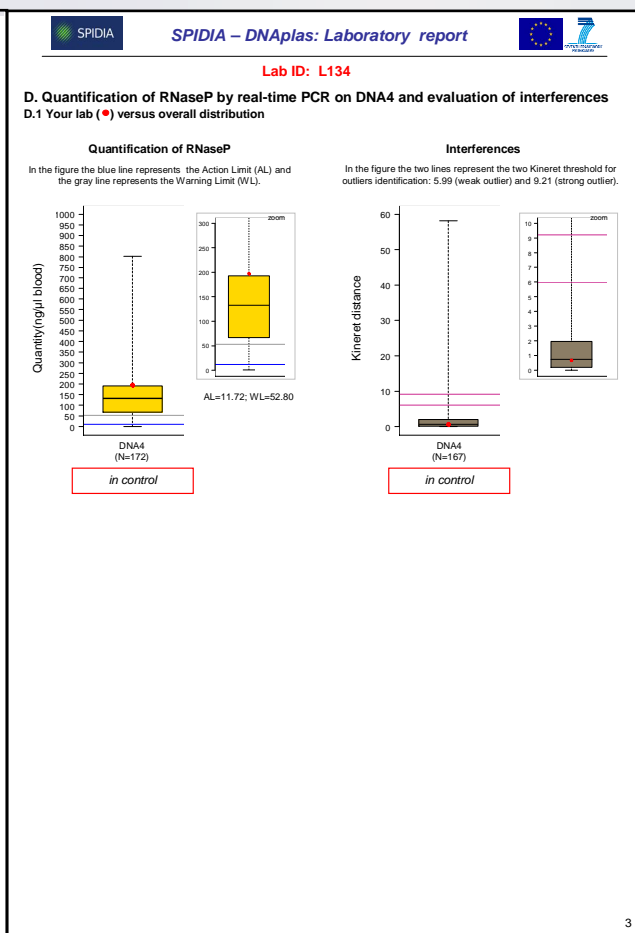
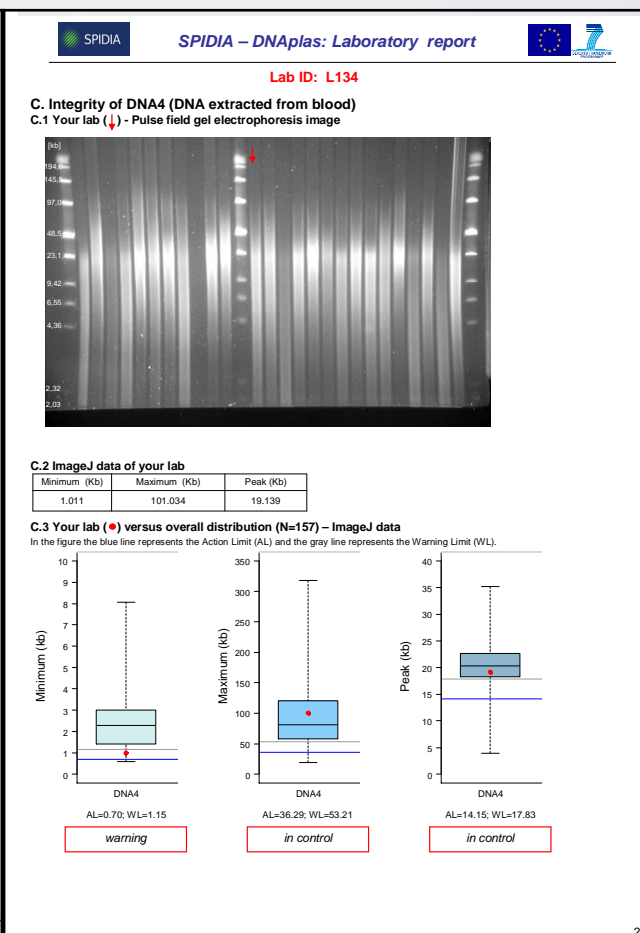
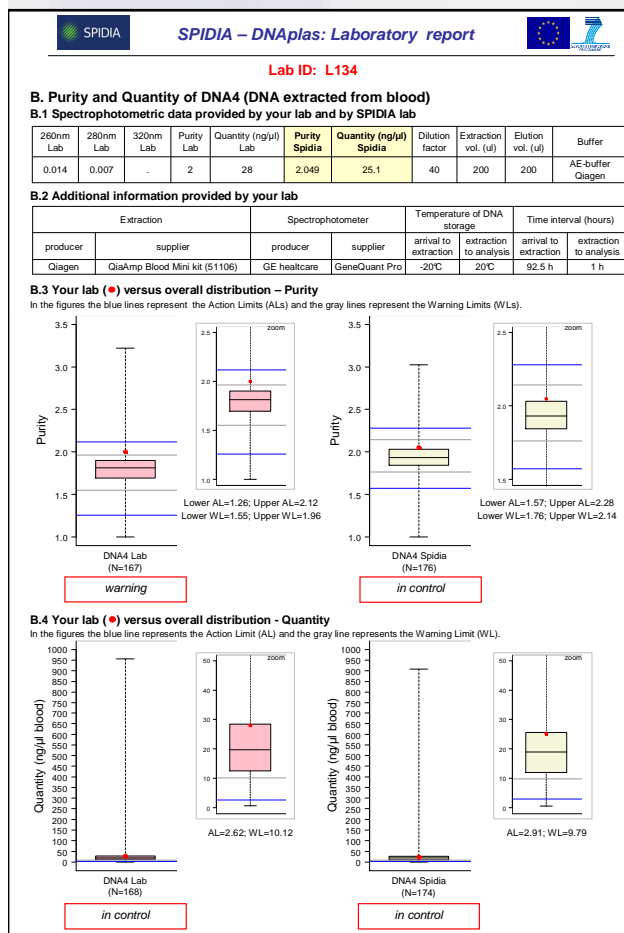
Evidence Based Guidelines

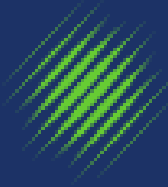
Examples Blood DNA & RNA, Plasma ccfDNA



- Led by Prof. Pazzagli (Univ. Florence), supported by the EFCC
- Phase 1 Trials - Laboratories used their workflows & tools
- Phase 2 Trials - Laboratories use SPIDIA's optimized workflows
- Guidelines / Technical Reports Development - CEN

SPIDIA Trials	No. of Participants (29 countries)	Participants who sent NA samples back	Percentage of NA samples sent back
Blood RNA	102	93	91 %
Blood DNA	130	121	93 %
Plasma DNA	67	62	93 %
Total	299	276	92 %





- Trainings, Workshops
- Newsletter (subscribe at www.sidia.eu)
- Scientific publications – 3 papers published, 6 manuscript submitted
- Co-operation with international initiatives and organizations
 - NCI / OBBR
 - Biospecimen Research Network (BRN)
 - Cancer Human Biobank (caHUB)
 - CLSI
 - EFCC
 - BBMRI
 - CD-Society (Austria)
 - m4 Cluster (Munich)

Standardization and Improvement of Generic Pre-analytical Tools and Procedures for *In Vitro* Diagnostics

■ **SPIDIA**

- Project Facts & Goals
- New Technologies & Tools
- Pan-European Guidelines & Dissemination
- **Evaluation of PAXgene Tissue**
 - PAXgene Tissue System & Workflow
 - Evaluation studies



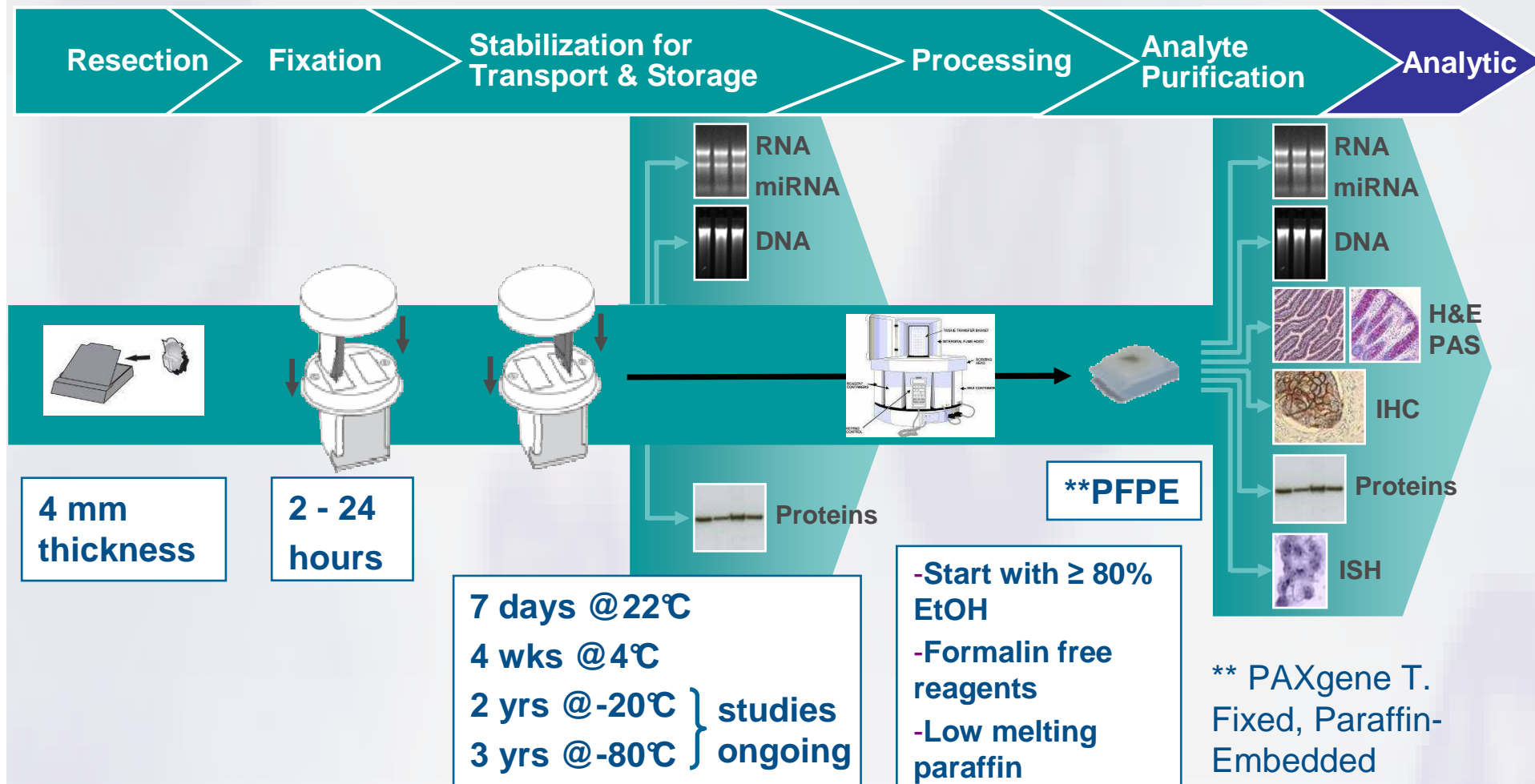
Tissue Preservation

Stabilization of Morphology and Biomolecules

- **Development began in 2007:**
 - **>1,500 compounds and combinations screened**
- **Technology requirements**
 - **Histomorphology must be equivalent to FFPE tissue**
 - **RNA, DNA, miRNA must be preserved and of high quality**
- **Two-reagent system finalized in 2009**
 - **Fixation and stabilization reagents, both formalin-free**
 - **Evaluation within Spidia ongoing**
 - **>8,000 tissue samples tested to date**
- **First collection device**
 - **Available as *PAXgene Tissue Container**
 - **Container with two chamber one closure**

* For Research Use Only

*PAXgene Tissue Workflow



* For Research Use Only

PAXgene Tissue System Components

PAXgene Tissue Container



- Tissue Fixation
- Tissue Stabilization
- Process Standardization
- Formalin-Free

PAXgene Tissue Kits



RNA



RNA&miRNA



DNA

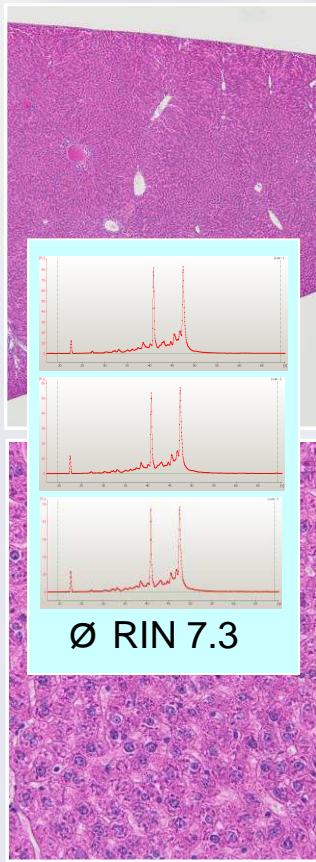
- **PAXgene Tissue Supplementary Protocols**
 - RNA/DNA or miRNA purification from microdissected tissue
 - Purification of full-length proteins from PFPE samples

For research use only. Not for use in diagnostic procedures. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

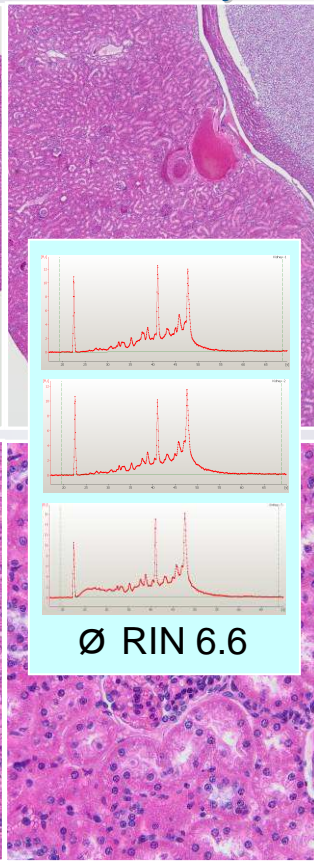
Morphology & RNA Preservation in Rat PFPE Tissue Samples

4 hours fixation, 7 days stabilization at 22°C

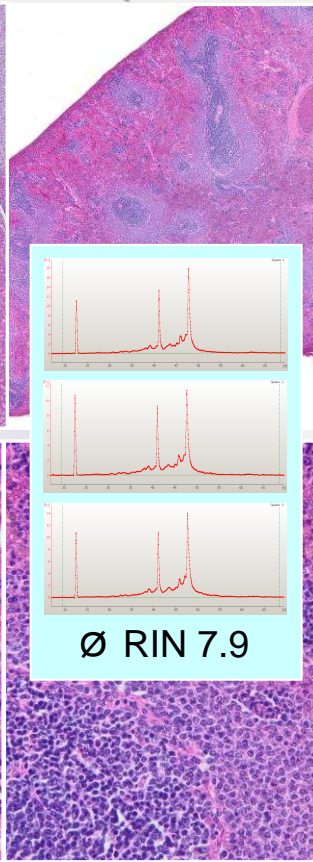
Liver



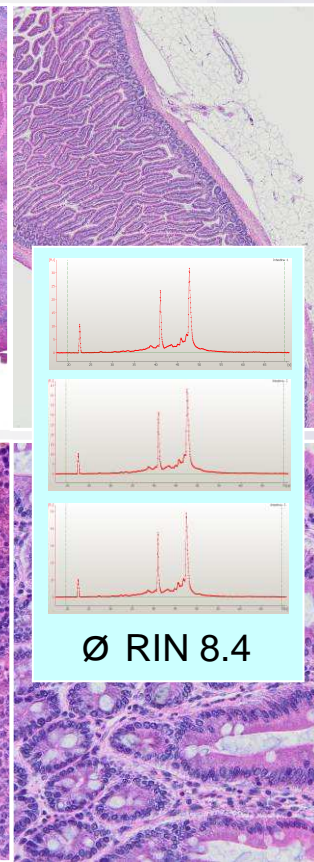
Kidney



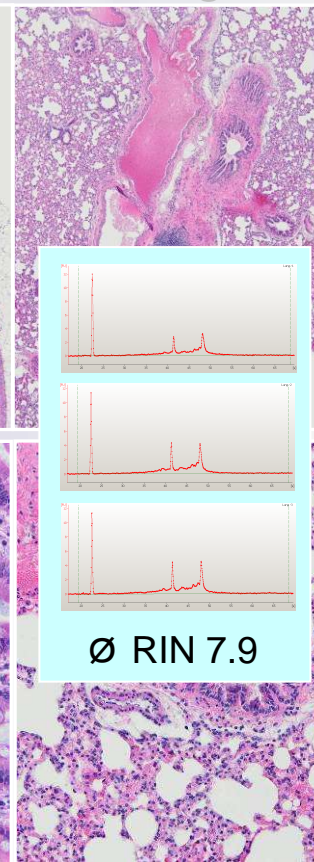
Spleen



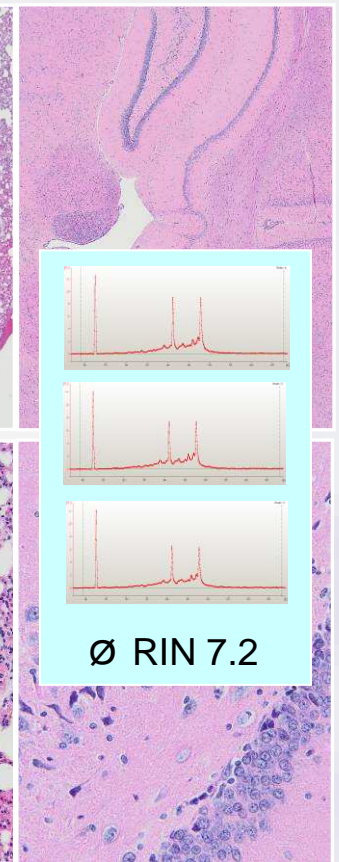
Intestine



Lung



Brain

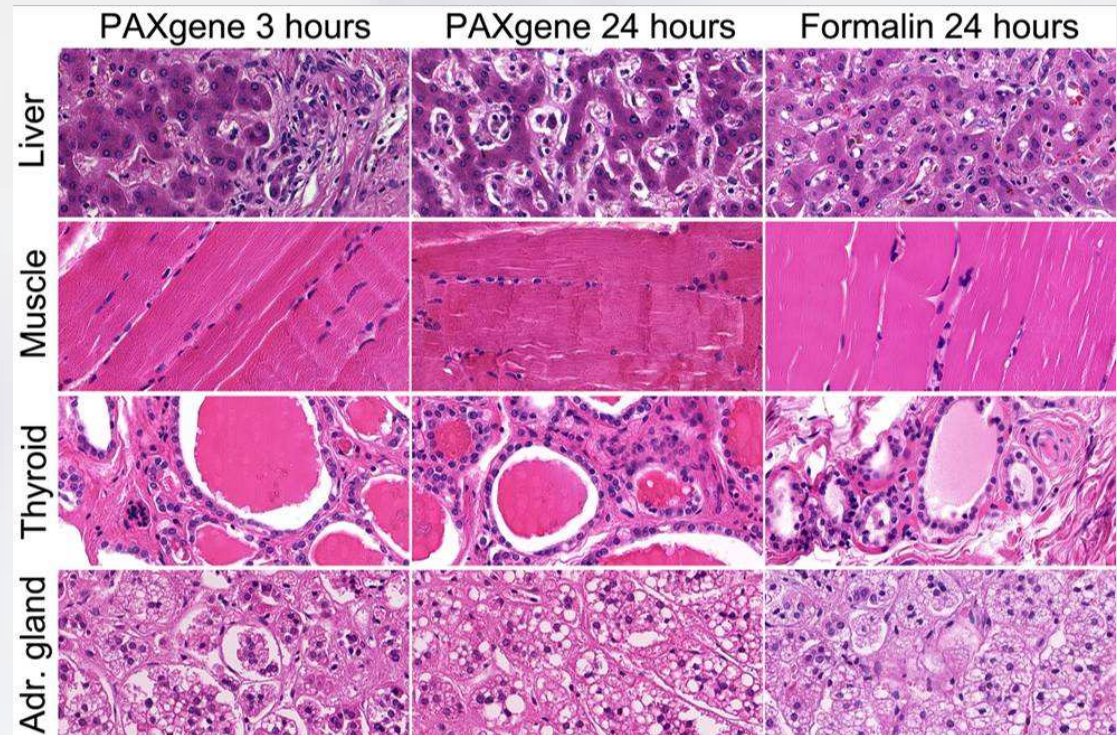


Morphology Preservation in Human Samples

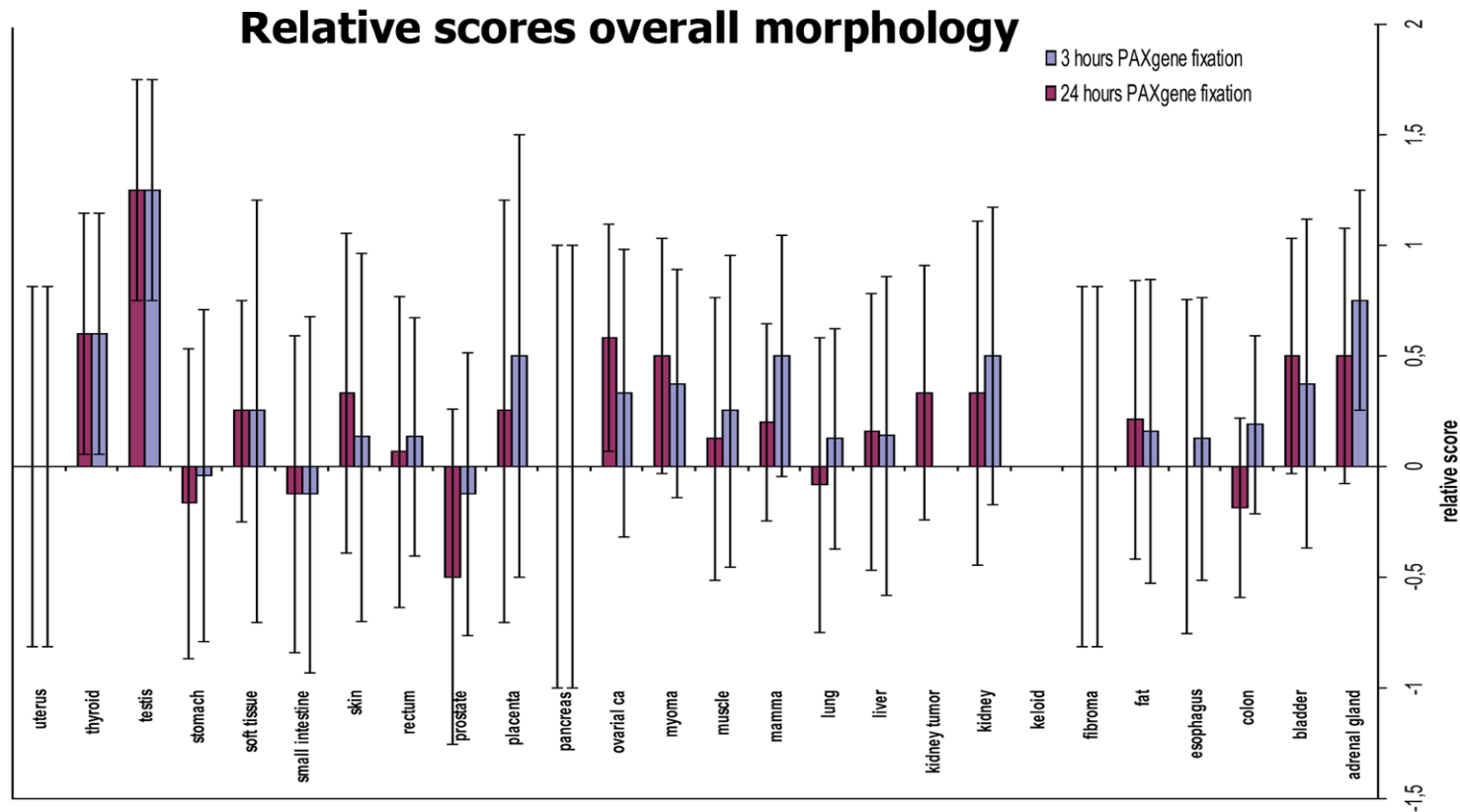
Histological Assessment of PAXgene Tissue Fixation and Stabilization Reagents

Kap M. *et al.*, PLoS ONE 6(11): e27704 (2011)

- 26 human tissue types
- Mirrored samples FFPE and PFPE
- Evaluation by virtual microscopy
- 4 pathologists from three institutes
- Scoring system -2 to +2
 - nuclear details
 - cytoplasmic details
 - membrane details
 - contrast
 - overall impression



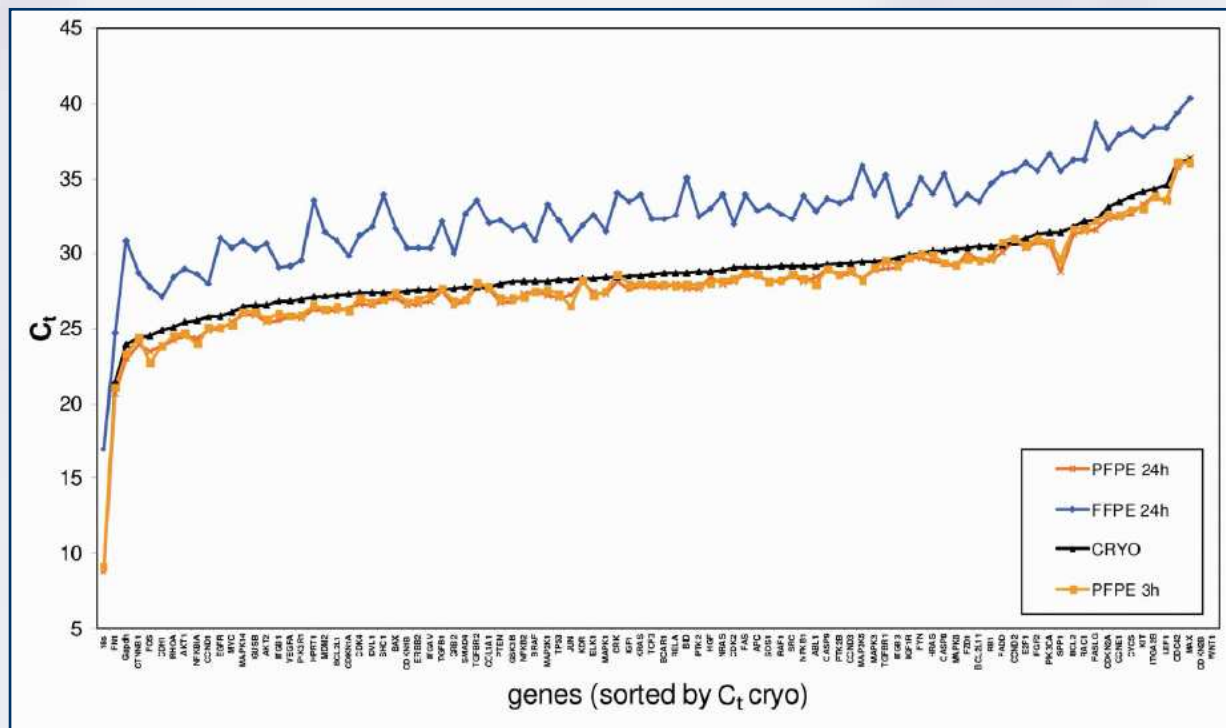
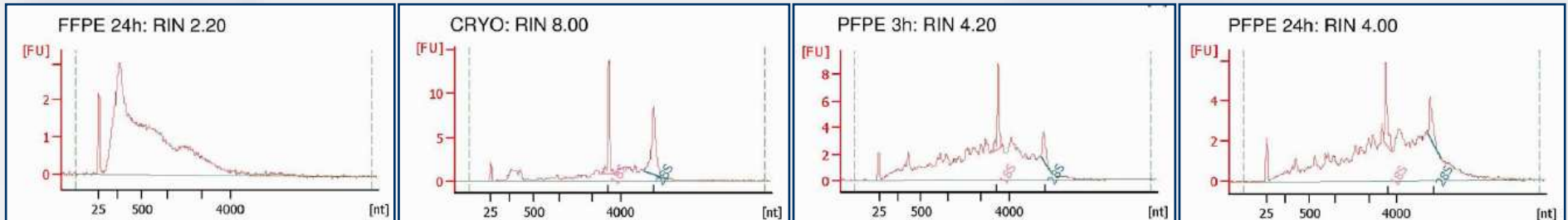
Kap *et al.*

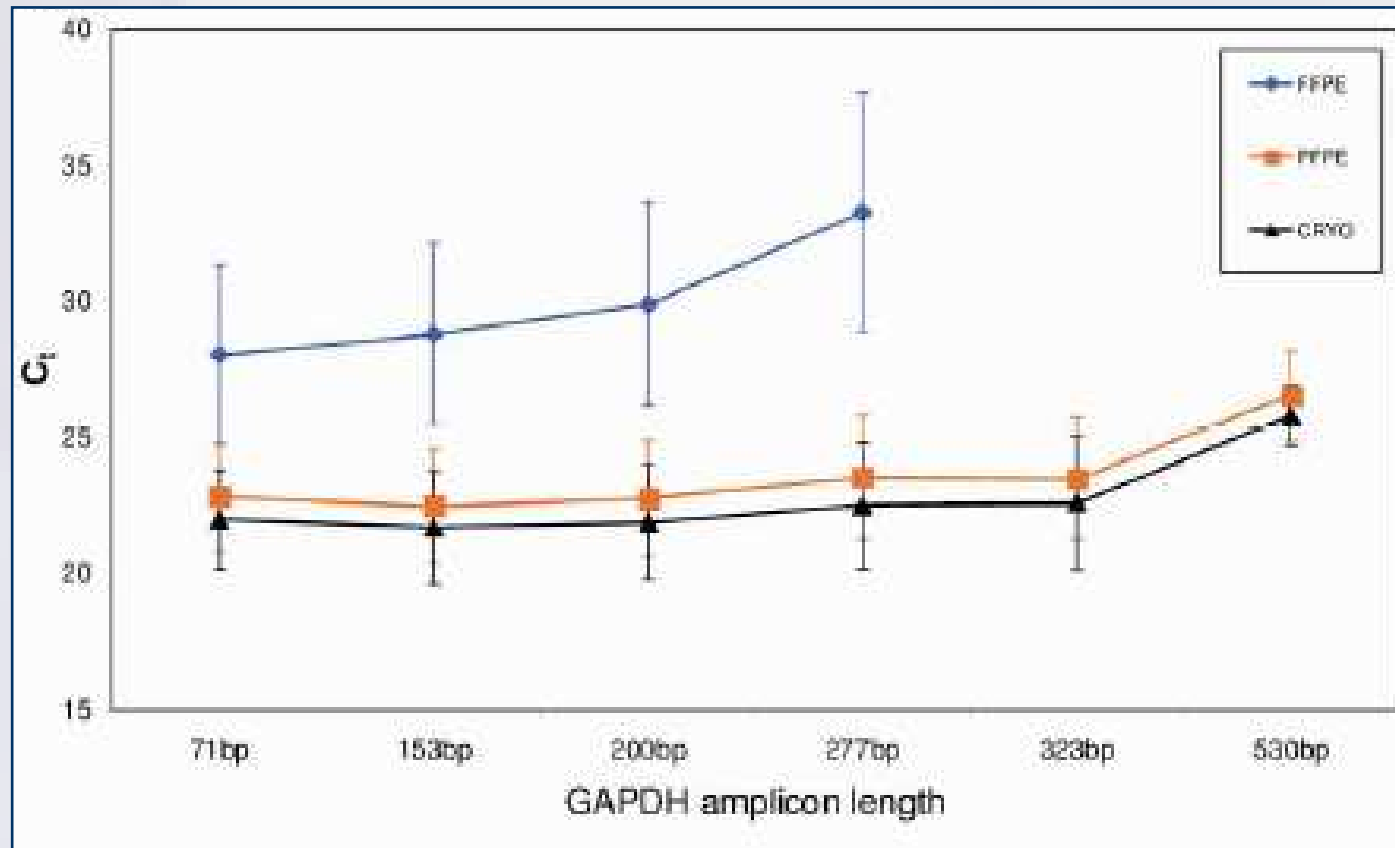


"morphology of PAXgene-fixed paraffin embedded tissue was well preserved"

"in general results obtained with PAXgene-fixed tissue are comparable to those of formalin-fixed tissue"

Liver sample: Cryo, PAXgene tissue and formalin fixed



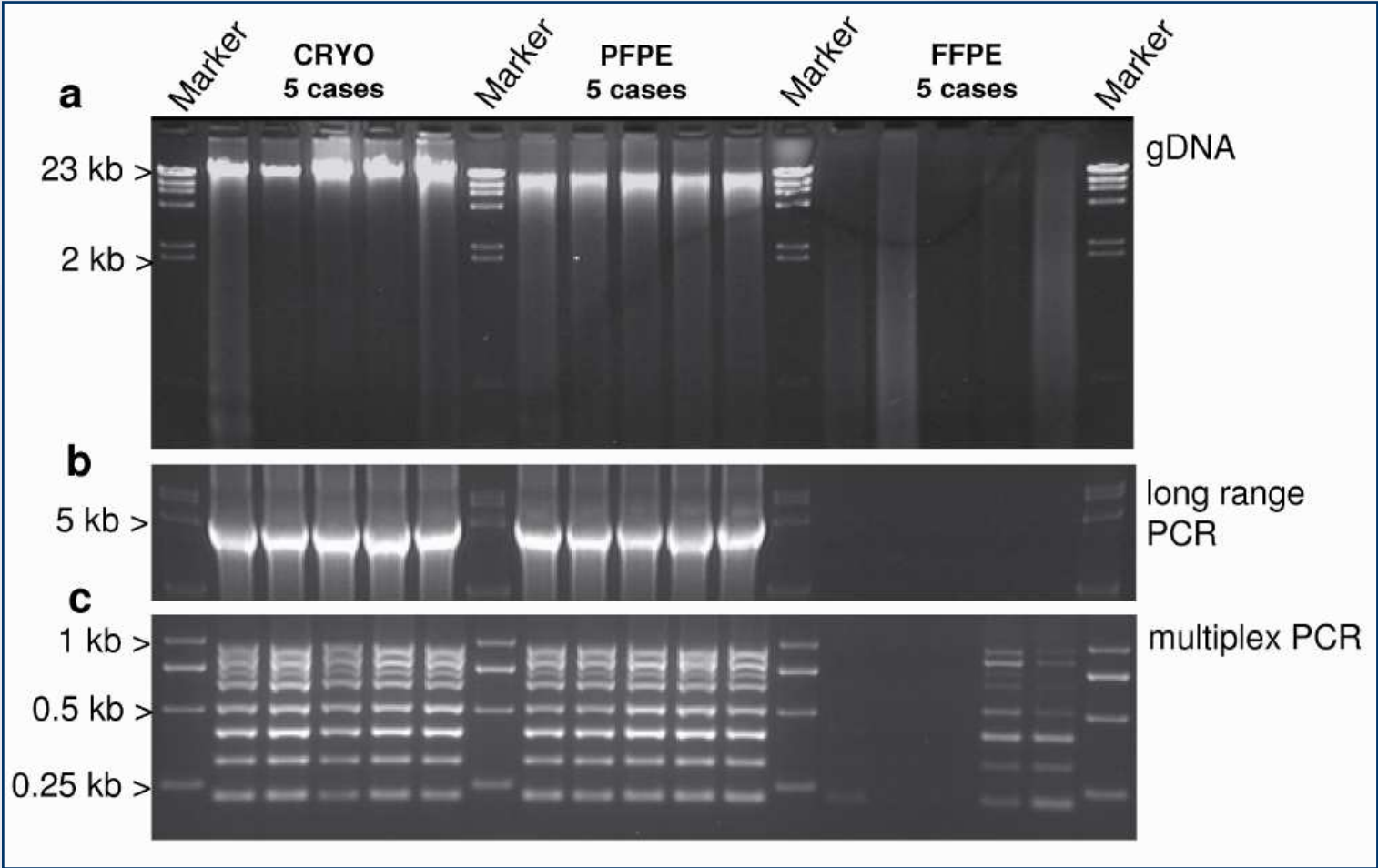


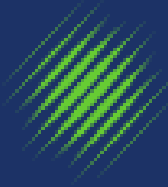
Viertler C. *et al.*,
accepted for
publication

- Ct summary of more than 800 RT-qPCR assays
- 45 human malignant and non-malignant tissue samples from different organs
- Samples fixed with PAXgene Tissue for 3 to 120 hours

DNA Preservation Human Clinical Samples

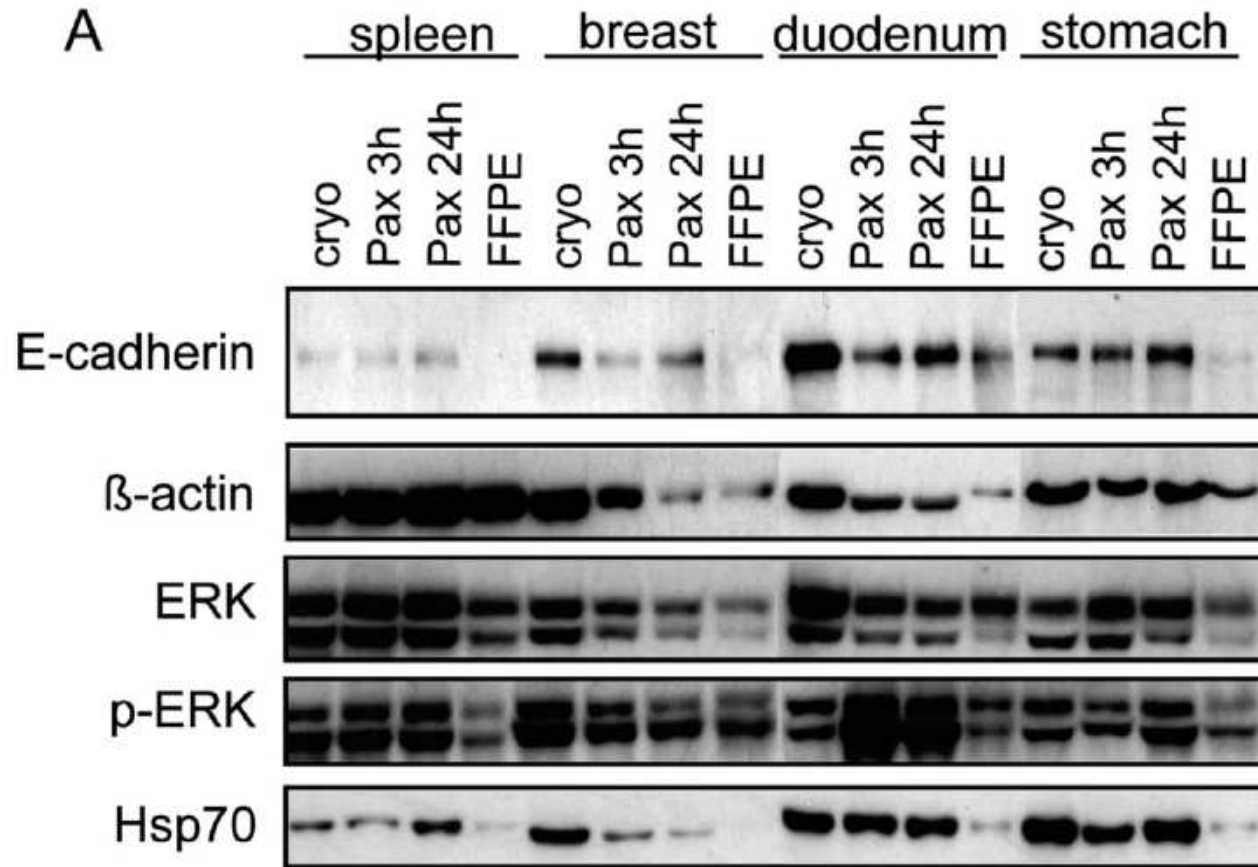
Colorectal cancer cases (CRC): Cryo, PAXgene tissue and formalin fixed





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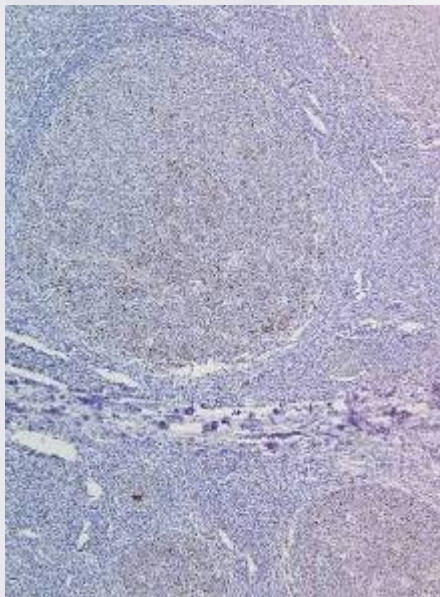
Protein Preservation in PFPE



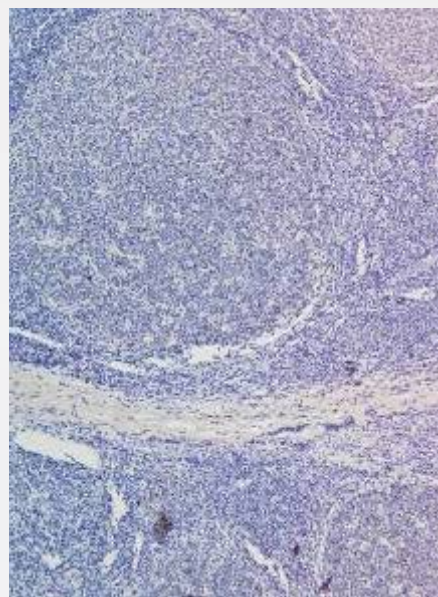
Proteins from PFPE tissues are non-degraded and immunoreactive

Ergin B. *et al.* Proteomic analysis of PAXgene fixed tissues. *J Proteome Res.* 9(10), 5188-96 (2010)

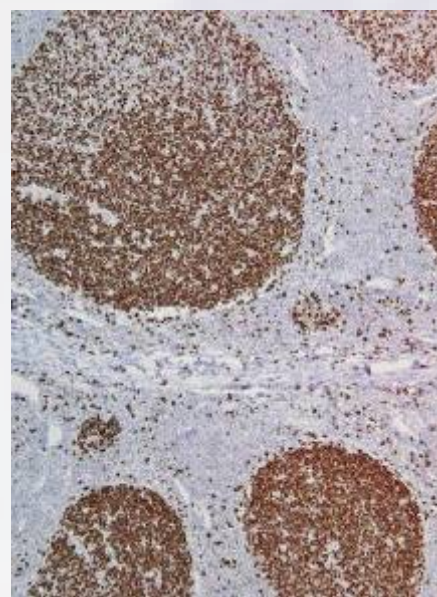
- PFPE human tonsil
- Ki-67, clone MIB-1
- Labelled streptavidin-biotin (LSAB) assay



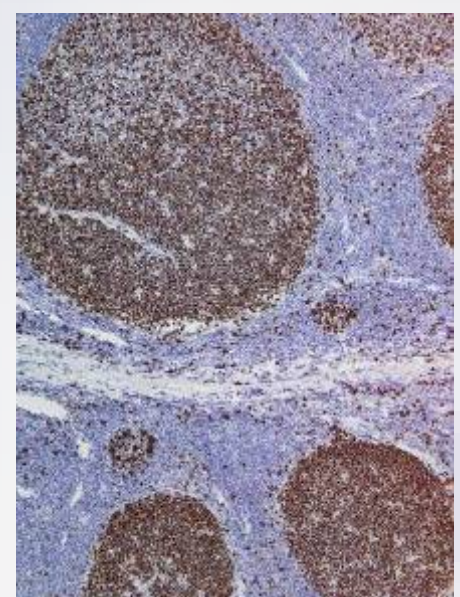
20 min at 98°C in
citrate buffer pH 6



no retrieval



20 min at 98°C in
Tris/EDTA buffer pH 9

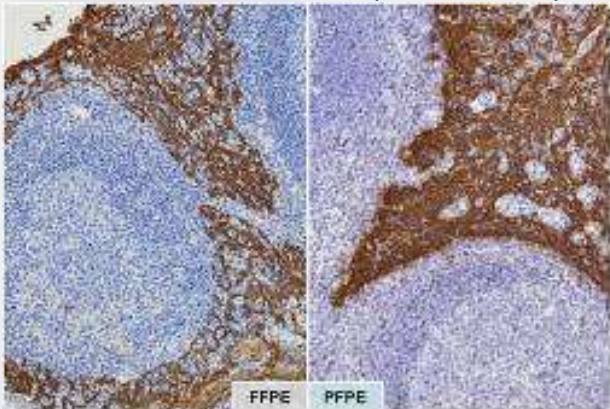


10 min at 70°C in
Tris/EDTA buffer pH 9



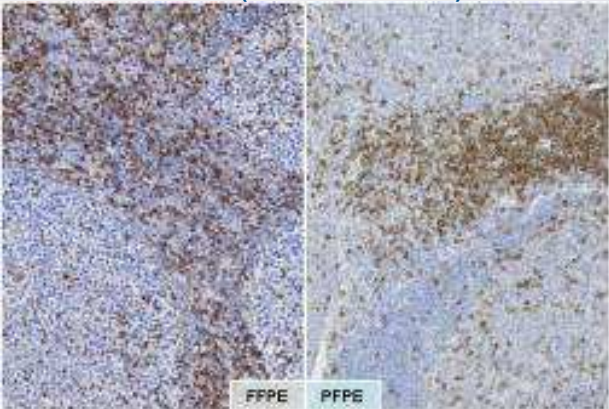
Immunohistochemistry Different Assays

Pankeratin CK (AE1/AE3)



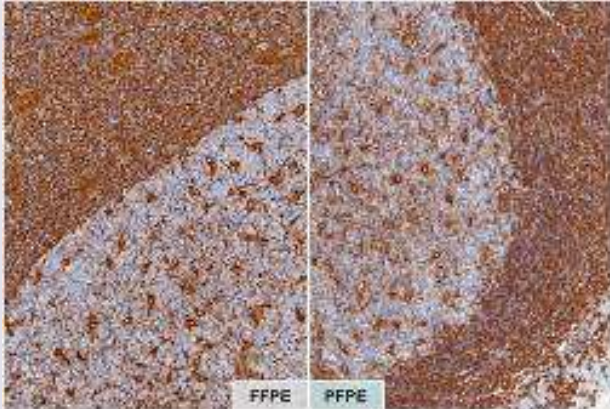
pH6, 20' 98°C | pH9, 10' 70°C

CD5 (CD5/54/F6)



pH9, 20' 98°C | pH9, 10' 98°C

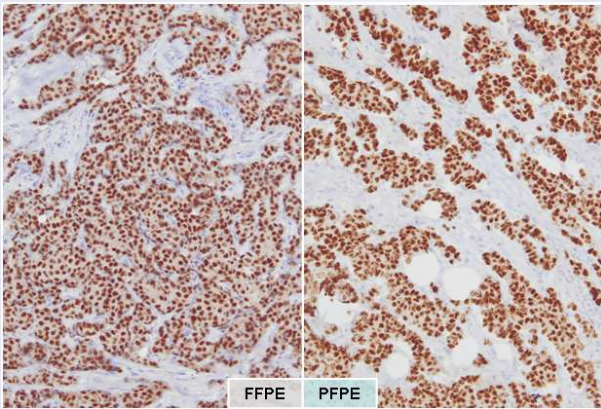
Vimentin (V9)



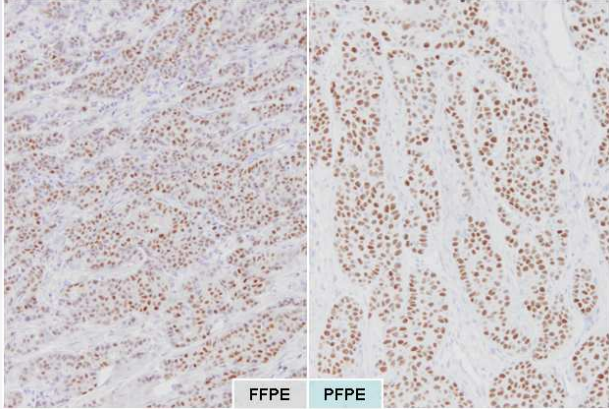
pH9, 20' 98°C | no HIER

Human Breast Cancer Cases

ERα (1D5)

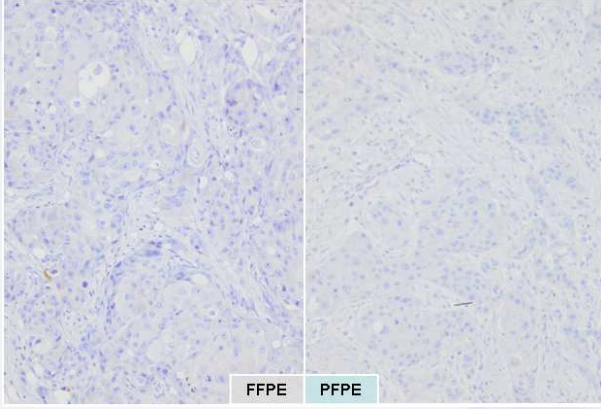


ERα (1D5)



pH9, 20' 98°C | pH9, 10' 98°C

ERα (1D5)



www.preanalytix.com

Acknowledgement

SPIDIA Consortium Members

- QIAGEN GmbH - Coordinator
- Medical University of Graz (*Prof. K. Zatloukal*)
- University of Florence (*Prof. M. Pazzagli*)
- CIRMMP Florence, CERM (*Prof. I. Bertini*)
- TATAA Biocenter
- PreAnalytiX GmbH
- DIAGENIC ASA
- Aros Applied Biotechnology
- Dako Denmark
- Biotechnology Inst. of Czech Academy of Science (*Prof. M. Kubista*)
- European Committee for Standardization (CEN)
- ImmunID Technologies
- Erasmus Medical Center Rotterdam (*Prof. P. Riegman*)
- Technical University Munich (*Prof. H. Hoefler, Prof. K. Becker*)
- Fondazione IRCCS Istituto Nazionale dei Tumori (*Dr. P. Verderio*)
- Novamen

Scientific Advisory Board

- *Prof. François Rousseau* (Univ. Laval, Quebec. CanGeneTest Network)
- *Dr. Roberta M. Madej* (CLSI)

Project Ethics Advisors

- *Dr. Anne Cambon-Thomsen* (CNRS, INSERM, Toulouse, France)
- *Dr. Ruth Chadwick* (ESRC Centre, Cardiff University, UK)



SPIDIA Consortium

Bi-Annual Meeting Berlin November 2011

