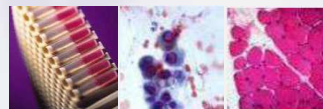




# Pre-Analytics & Biobanking



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Institute of Pathology  
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*EScoP, Basic Molecular Biology for Pathologists  
Graz, March 31 – April 4, 2014*



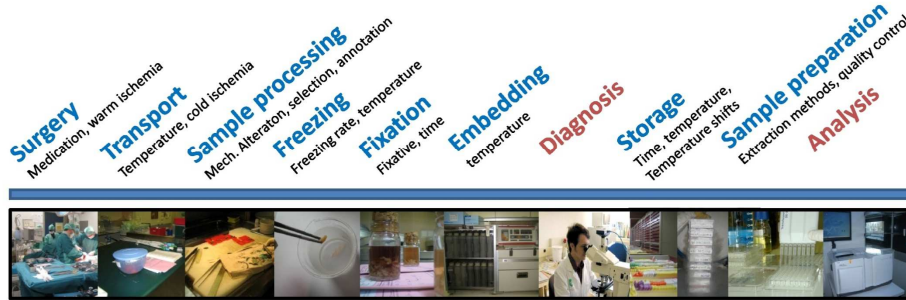
## Outline



- **Critical variables within the pre-analytical workflow of tissue samples**
- **Current status and future perspective of tissue preservation**
- **RNA quality control and gene expression analysis from (fixed and paraffin-embedded) tissue samples**
- **SPIDIA comparative studies PFPE, FFPE and cryopreserved samples**
- **Preservation of morphology, antigenicity, nucleic acids**
- **Conclusions**



# Pre-Analytical Workflow of Tissues Samples



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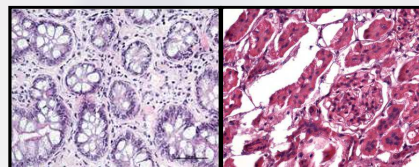
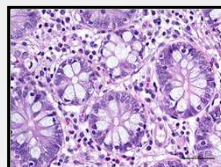
# Current status of tissue preservation



FFPE

Liquid nitrogen, RNAlater

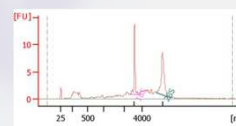
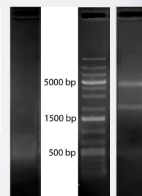
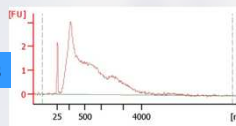
Morphology



Gold standard

Limited use

Nucleic acids



Limited use

Gold standard

Drawbacks

Impaired preservation of biomolecules  
Crosslinking

Impaired morphology preservation  
Logistics, costs

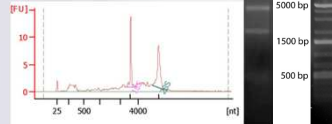
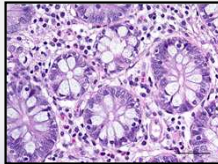
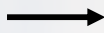
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## Future perspective of tissue preservation

One tissue specimen

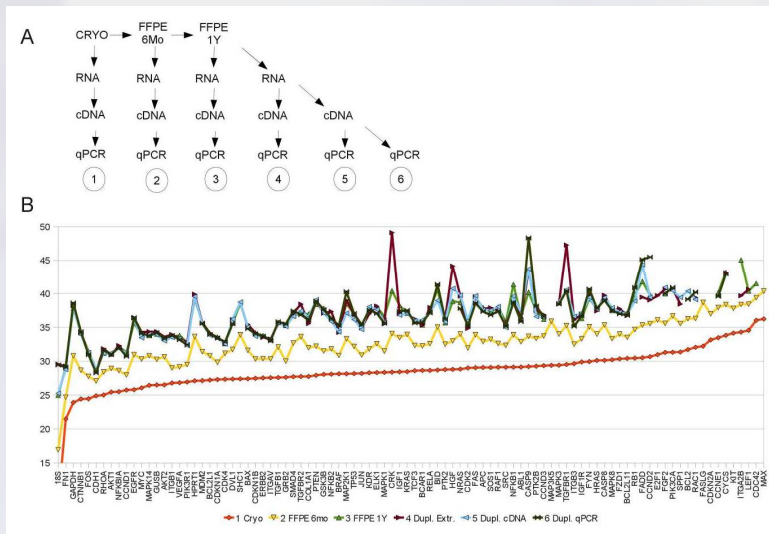
Morphology

Biomolecules

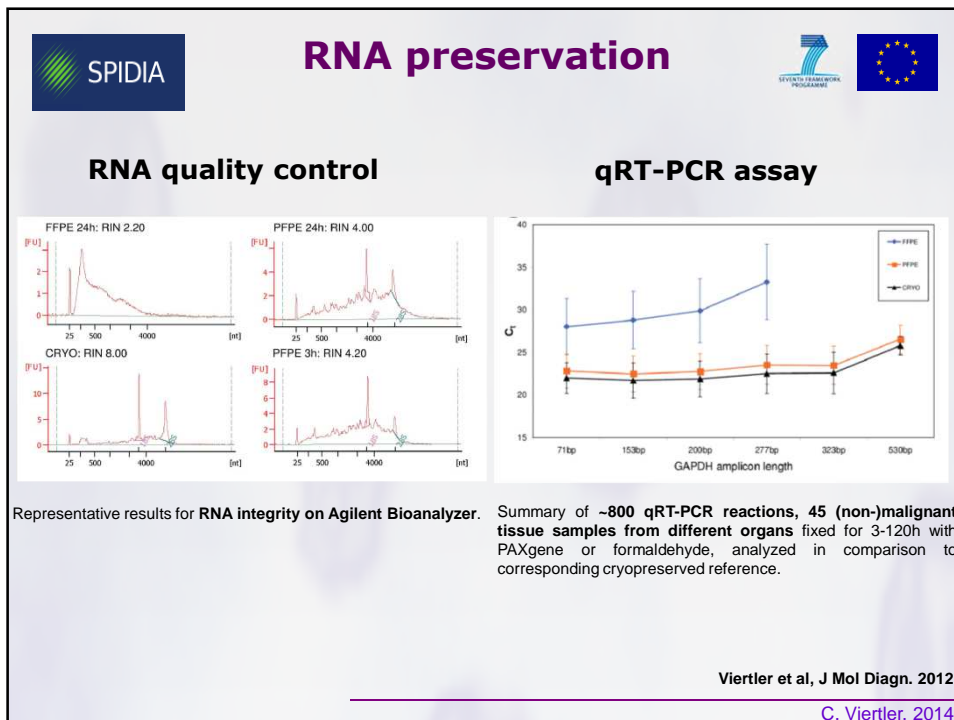
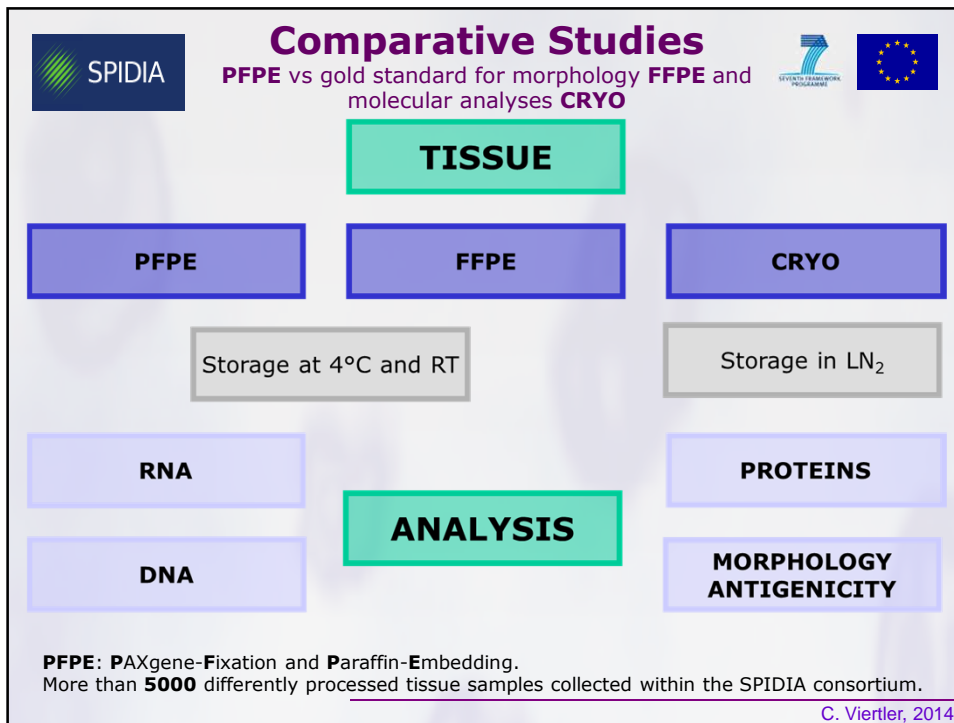


Histopathological analyses and molecular studies from the same tissue sample on a high-quality level.

## Gene Expression Analysis Fixation and storage effects

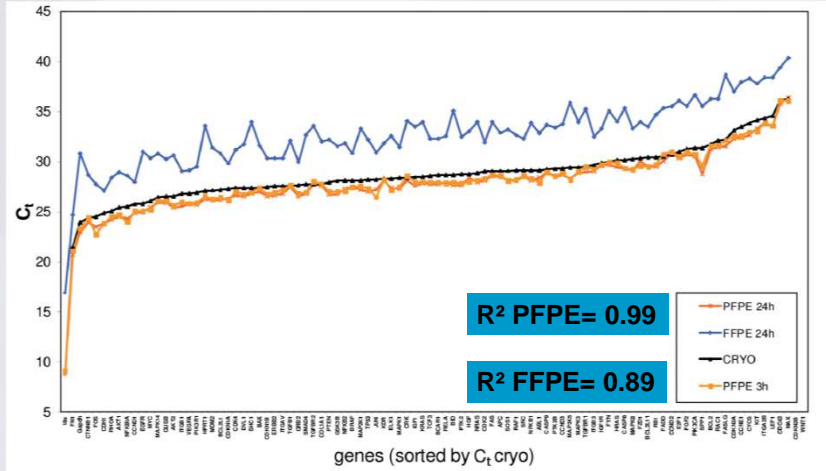


Fixation and storage introduces major gene-to-gene variations in qRT-PCR.





## Gene expression analysis PFPE vs FFPE, CRYO ref



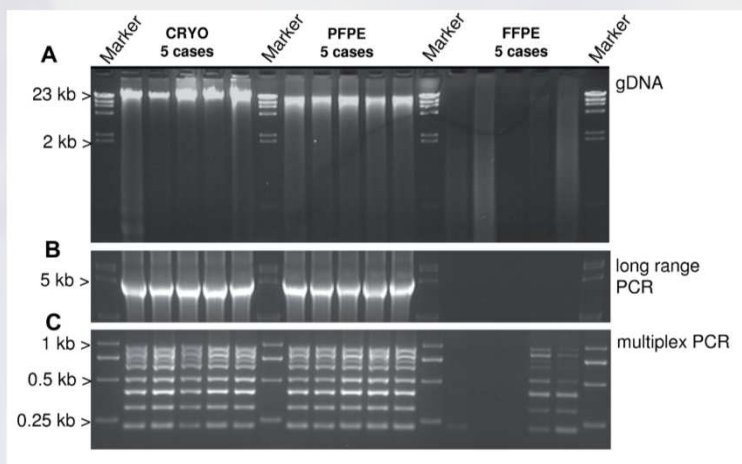
Viertler et al, J Mol Diagn. 2012

Gene expression analysis of 92 cancer pathway-associated and 4 endogenous control genes (18S, GAPDH, GUSB, HPRT1) by qRT-PCR on predefined TaqMan array "Human Molecular Mechanisms of Cancer" plate.

C. Viertler, 2014



## DNA integrity, long-range & multiplex PCR



Viertler et al, J Mol Diagn. 2012

FFPE, PFPE, and snap-frozen (CRYO) samples of 5 human colorectal cancer cases.

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## Conclusions



- **The quality and reliability of tissue-based molecular analyses can only be defined in the context of pre-analytical procedures**
- **Pre-analytical variables e.g. ischemia, preservation method, fixation time and storage conditions impact molecular analyses**
- **Established methods for RNA QC are well suited for frozen tissue but less reliable for (formalin-fixed and) paraffin-embedded tissues**
  - Additional QC recommended depending on the level of standardisation of the pre-analytical workflow
- **Documentation of the pre-analytical sample history is crucial for biobanking**
- **New alternatives to routine formalin fixation should be considered for:**
  - comprehensive tissue diagnostics, clinical trials, biomedical research
  - biomarker discovery programmes in a routine clinical setting
  - molecular studies whenever a collection of snap frozen samples is not possible
  - direct correlation of disease phenotypes with alterations of biomolecules

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## THANK YOU!



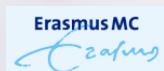
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