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

Current status of tissue preservation

**FFPE**
- Gold standard
- Limited use
- Impaired preservation of biomolecules
- Crosslinking

**Liquid nitrogen, RNAlater**
- Gold standard
- Limited use
- Impaired morphology preservation
- Logistics, costs

C. Viertler, 2014
Future perspective of tissue preservation

One tissue specimen + Biomolecules

Morphology

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.

Viertler, Kashafer et al; PLoS ONE 2013

C. Viertler, 2014
Comparative Studies
PFPE vs gold standard for morphology FFPE and molecular analyses CRYO

PFPE: PAXgene-Fixation and Paraffin-Embedding.
More than 5000 differently processed tissue samples collected within the SPIDIA consortium.

C. Viertler, 2014

RNA preservation

RNA quality control
qRT-PCR assay

Representative results for RNA integrity on Agilent Bioanalyzer.
Summary of ~800 qRT-PCR reactions, 45 (non-)malignant tissue samples from different organs fixed for 3-120h with PAXgene or formaldehyde, analyzed in comparison to corresponding cryopreserved reference.


C. Viertler, 2014
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.


DNA integrity, long-range & multiplex PCR


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

THANK YOU!