SPIDIA Formalin-free Tissue Fixation for Multimodal Biomarker Research Analysis

PreAnalytiX A QIAGEN / BD Company

SPIDIA: Standardization and improvement of generic pre-analytical tools and procedures for in-vitro diagnostics

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Background

While tissue fixation with formalin preserves morphology, it leads to crosslinking and chemical modification of biomolecules. Since chemical modifications cannot be completely reversed, biomolecules isolated from FFPE tissue are of limited use for molecular analysis. The PAXgene® Tissue System (PAXgene) is a non-crosslinking technology for fixation and stabilization of tissue specimens. PAXgene-fixed, paraffin-embedded (PFPE) tissue sections are suitable for conventional histochemical and immunohistochemical staining as well as for extraction of unmodified biomolecules such as nucleic acids, proteins, and phosphoproteins (Figure 1).

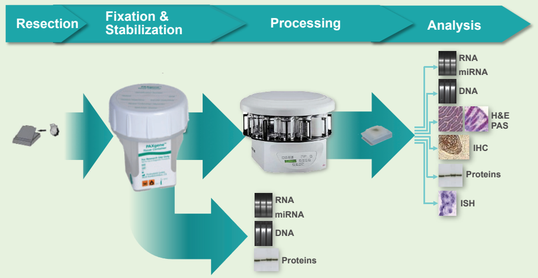

Figure 1. PAXgene Tissue workflow

Material & Methods

Tissue Research Specimens

- Human liver, cystadenoma (benign)
- Human ovarian, prostate and breast cancer (malignant)
- Divided and fixed in formalin, PAXgene, or cryo-preserved (See Figure 2.)

Tissue Morphology Analysis of FFPE and PFPE Sections

- H&E staining
- ERα and PR immunohistochemistry (IHC) staining
- HER2 gene amplification determined by SPoT-Light® HER2 CISH kit (Life Technologies)

RNA Analysis

- RT-qPCR: TaqMan® Array Gene Signature 96-well plates (Applied Biosystems); RT² Profiler™ PCR Arrays (SABiosciences)

DNA Analysis

- Agarose gel electrophoresis
- Long-range PCR Kit (QIAGEN)
- Multiplex PCR Kit (QIAGEN)

Protein and Phosphoprotein Analysis

- Western blot

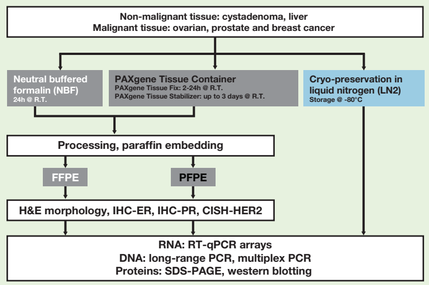

Figure 2. Study design

Results

- **Morphology** was preserved in PFPE liver and breast cancer tissue similarly to FFPE (Figure 3).
- The staining intensity of **IHC** for estrogen and progesterone receptors in breast cancer samples were comparable between PFPE and FFPE tissue (Figure 4).
- The number and size of dots in the HER2 **CISH** assay were comparable between PFPE and FFPE samples (Figure 5).
- There was a high correlation between **gene signatures** of PFPE and cryo-preserved liver and breast cancer samples in two different TaqMan arrays (R^2=0.97 and 0.92) (Figures 6A, B).
- There was poor correlation between **gene signatures** of FFPE and cryo-preserved liver and breast samples in both TaqMan arrays (R^2=0.79 and 0.65) and major gene-to-gene variations within FFPE tissue as compared to PFPE and cryo-preserved specimens. (Figures 6A and 6B).
- **DNA** from cryo-preserved and PFPE tissue was of high molecular weight and performed well in PCR (Figures 7A, B, C).
- **DNA** from FFPE was highly degraded and failed in most PCR assays (Figures 7A, B, C).
- **Western blot** signal intensities were similar between PFPE and cryo-preserved tissue for the phosphoproteins p-PTEN, p-Akt, p-GSK3β, p-PRAS40, p-p44/42 MAPK, and p-p38 (Figure 8).
- **Western blot** signals from FFPE tissue were weaker than signals from the cryo-preserved reference material (Figure 8).

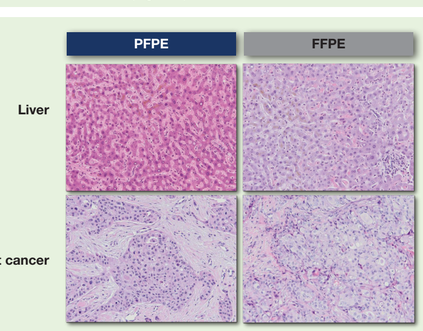

Figure 3. H&E morphology of mirrored samples FFPE and PFPE

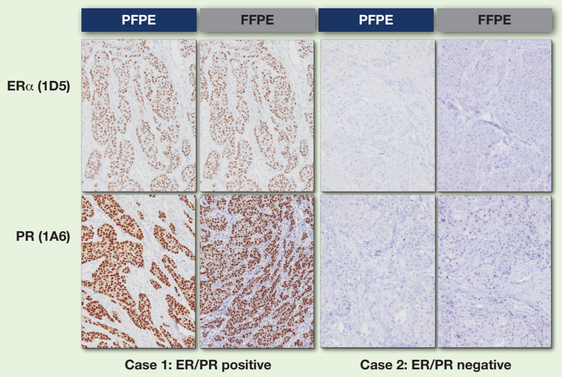

Figure 4. ERα-, PR- Immnohistochemistry (IHC) staining on two cases of mirrored breast cancer samples.

Estrogen receptor alpha (clone 1D5) and progesteron receptor immunostaining (clone 1A6); epitope retrieval in Tris/EDTA buffer pH 9 for 10 and 20 min at 98°C, for PFPE and FFPE sections, respectively.

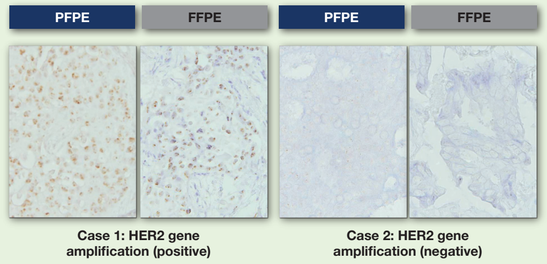

Figure 5. Chromogenic *in situ* hybridization (CISH) of human HER2 gene on two cases of mirrored breast cancer samples, using the SPoT-Light HER2 CISH Kit from Life Technologies on sections of 6 µm thickness. Pretreatment of FFPE sections by heating for 15 min at 99°C and 5 min enzymatic digestion. No pretreatment of PFPE sections; counterstained with hematoxylin.

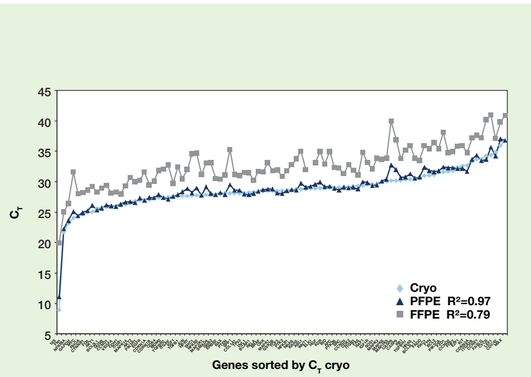

Figure 6. Gene expression analysis in RT-qPCR arrays.

Gene expression of 92 or 84 cancer pathway-associated genes from corresponding PFPE, FFPE, and cryo-preserved human liver and human breast cancer cases analyzed by RT-qPCR on predefined (A) TaqMan array "Human Molecular Mechanisms of Cancer" plate and (B) RT² Profiler PCR Array "Human epithelial to mesenchymal transition" plate.

RT-qPCR arrays show high correlation of gene signature in PFPE (▲) and cryo-preserved (♦) samples (R^2=0.97 and R^2= 0.92), whereas FFPE (■) samples show a decreased correlation (R^2=0.79 and R^2= 0.65) and major gene-to-gene variations.

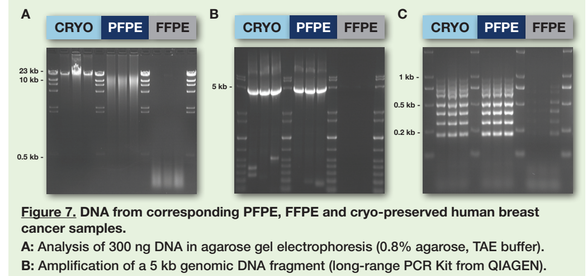

Figure 7. DNA from corresponding PFPE, FFPE and cryo-preserved human breast cancer samples.

A: Analysis of 300 ng DNA in agarose gel electrophoresis (0.8% agarose, TAE buffer).
B: Amplification of a 5 kb genomic DNA fragment (long-range PCR Kit from QIAGEN).
C: Multiplex PCR of eight different genomic DNA fragments ranging from 222 to 955 bp (QuantiTect® Multiplex PCR Kit from QIAGEN).

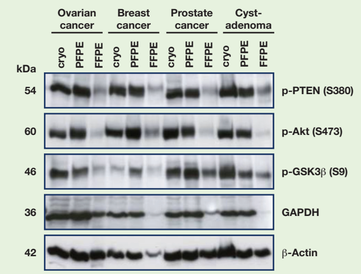

Figure 8. Preservation of phosphoproteins in cryo- preserved, PFPE and FFPE human tissue samples.

Human ovarian, breast and prostate cancer and cystadenoma specimens were equally divided into three samples and either snap frozen (cryo), PAXgene-fixed (24 h) and paraffin-embedded (PFPE) or formalin-fixed (24 h) and paraffin-embedded (FFPE). After protein extraction, 15 µg protein per lane was separated by SDS-PAGE and western blot analysis was performed using indicated antibodies.

Conclusions

- Morphology and antigenicity is preserved in PFPE tissues and is comparable to morphology in freshly prepared FFPE samples.
- Unlike FFPE tissue, chemically unmodified DNA, RNA, and proteins can be isolated from PAXgene Tissue fixed samples.

Summary

Using PAXgene Tissue, multimodal analysis for biomarker research can be performed on the same sample used for morphological analysis.

Disclaimer

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.

Acknowledgments

Surgical resected tissue was collected by commercial providers with prior written informed consent by the patient.
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