Preservation of Morphology and Biomolecules Within Tissue Stored for Three Years at -80°C in PAXgene Tissue Stabilizer Reagent

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Background

For preservation of biomolecules in tissue, current standard practice is to snap freeze tissue specimens in liquid nitrogen (LN2) and store them at -80°C. Under these conditions, however, tissue morphology is compromised. If examination of tissue morphology by histochemical or immunohistochemical means is required, formalin fixation and embedding in paraffin (FFPE) is standard practice.

The PAXgene Tissue System (PAXgene) is a non-crosslinking formalin substitute consisting of a tissue fixative and a tissue stabilization reagent. After fixation and transfer into the stabilizer, tissues can be stored at -80°C to preserve morphology as well as all biomolecules. After storage, biomolecules can be isolated directly from PAXgene stabilized tissue samples or they can be processed and paraffin-embedded (PFPE) for analysis of morphology and biomolecules.

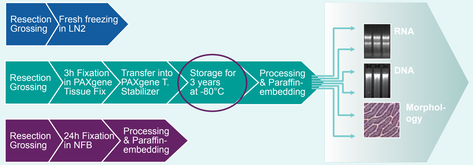
Materials and Methods

Rat tissue specimens were fixed in PAXgene Tissue Fixative for 3 hours, transferred into PAXgene Tissue Stabilizer and stored in the Stabilizer at -80°C. After 3 years of storage in the Stabilizer, specimens were processed and embedded in paraffin. Quality of morphology was analyzed by H&E staining, and nucleic acids were purified from sections of paraffin embedded tissue using the corresponding PAXgene Tissue kits. For comparison, mirrored rat specimens were fixed for 24 hours with neutral buffered formalin or fresh frozen in liquid nitrogen (Fig. 1, Tab. 1).

Table 1: Nucleic acids preparation and amplification kits

	Fresh freezing in liquid nitrogen (LN2)	PAXgene Tissue PAXgene Tissue Fix: 3h @ R.T. PAXgene Tissue Stabilizer: 3 years at -80°C	Neutral buffered formalin (NBF) 24h @ R.T.
RNA incl. miRNA purification	miRNeasy Mini (QIAGEN)	PAXgene Tissue miRNA (PreAnalytiX)	miRNeasy® FFPE (QIAGEN)
DNA purification	QIAamp Mini (QIAGEN)	PAXgene Tissue DNA (PreAnalytiX)	QIAamp® FFPE (QIAGEN)
RT-qPCR assays	QuantiTect® SYBR® Green RT-PCR kit (QIAGEN)		
qPCR assays	Rotor-Gene SYBR Green PCR kit (QIAGEN)		

Figure 1: Workflow



Results

Morphology in PAXgene Tissue specimens stored for 3 years at -80°C was well preserved and comparable to freshly prepared FFPE specimens. Morphology in fresh frozen specimens, embedded in OCT medium, was less well preserved and exhibited artifacts including shrinkage or swelling (Fig. 2).

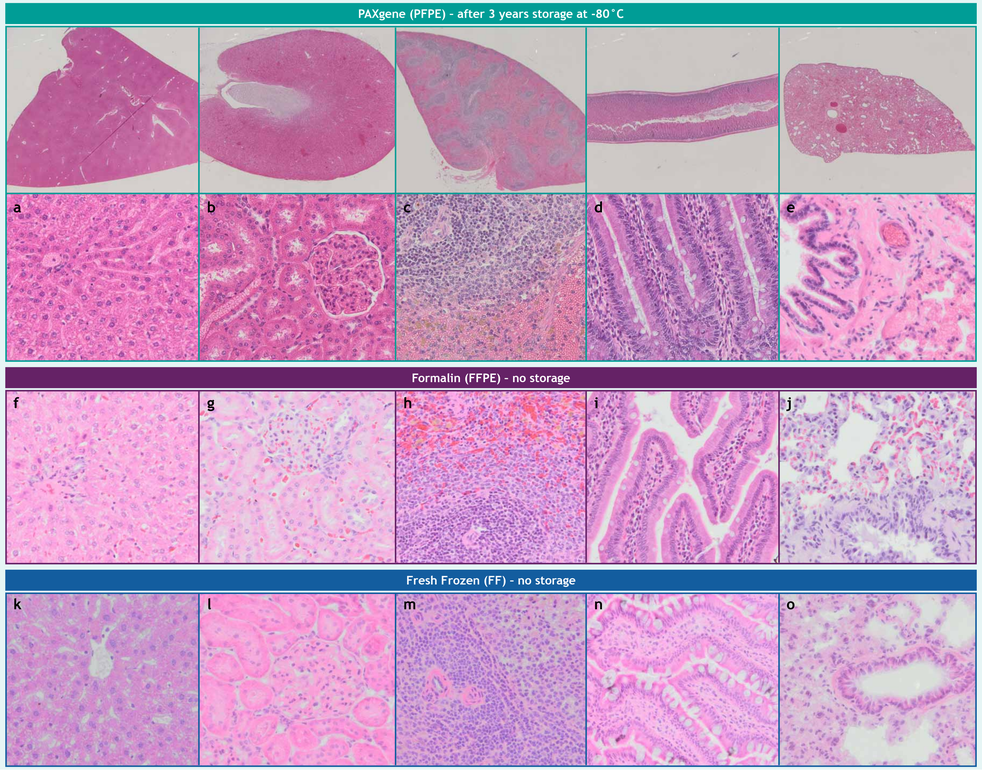
Nucleic acids of high molecular weight and integrity could be recovered from both fresh frozen and PFPE tissue with RNA integrity scores between 6 to 8 (Fig. 3) and DNA fragments predominantly in the range of 10 to 23 kb in length (Fig. 5).

In contrast, RNA integrity scores for FFPE tissue varied between 2 to 8 and DNA appeared as a smear on the gel electrophoresis with fragment lengths between 0.5 to 10 kb (Fig. 3 and Fig. 5).

Performance in qRT-PCR (Fig. 4) and qPCR (Fig. 6) was comparable between nucleic acids isolated from fresh frozen and PFPE tissue with constant delta- C_T values ($\Delta C_T = C_{T\,[PFPE]} - C_{T\,[FF]}$) between -1 to 1.5 regardless of amplicon length.

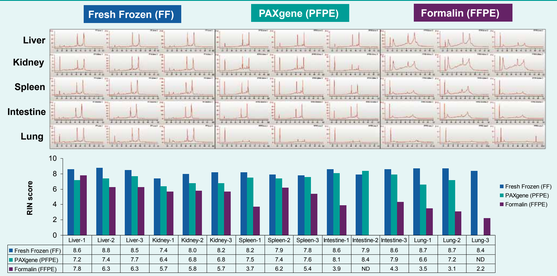
In contrast, delta-C_T values for RNA from FFPE tissue ($\Delta C_T = C_{T\,[FFPE]} - C_{T\,[FF]}$) showed inhibition of qRT-PCR in an amplicon length dependent way with delta-C_T values from 3 to 13 (Fig. 4). This amplicon size-dependent inhibition was less apparent in DNA amplification where delta-C_T values ranged between 4 and 6.

Figure 2: Morphology of rat tissue specimens preserved three ways



Morphology of H&E stained rat liver (a,f,k), kidney (b,g,l), spleen (c,h,m), small intestine (d,I,n) and lung (e,j,o). PAXgene Tissue (a-e) fixed/stabilized samples were stored for 3 years at -80°C within Stabilizer before processing and paraffin-embedding; formalin-fixed (f-j) and fresh frozen (k-o) samples without storage; original magnifications x 400.

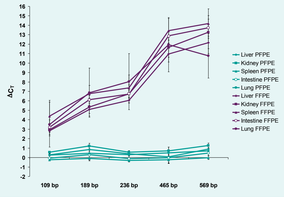
Figure 3: RNA Integrity of RNA obtained from rat specimens preserved three ways



	Liver-1	Liver-2	Liver-3	Kidney-1	Kidney-2	Kidney-3	Spleen-1	Spleen-2	Spleen-3	Intestine-1	Intestine-2	Intestine-3	Lung-1	Lung-2	Lung-3
Fresh Frozen (FF)	8.6	8.8	8.5	7.4	8.0	8.2	8.2	7.9	7.8	8.6	7.9	8.6	8.7	8.7	8.4
PAXgene (PFPE)	7.2	7.4	7.7	6.4	6.8	6.8	7.5	7.4	7.6	8.1	8.4	7.9	6.6	7.2	ND
Formalin (FFPE)	7.8	6.3	6.3	5.7	5.8	5.7	3.7	6.2	5.4	3.9	ND	4.3	3.5	3.1	2.2

Analysis of total RNA from rat tissue fresh frozen (FF), formalin- or PAXgene Tissue fixed and paraffin-embedded (FFPE or PFPE) by microcapillary electrophoresis.

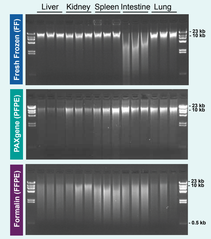
Figure 4: RNA performance in RT-qPCR



Reverse transcription and amplification using 10 ng RNA each from rat tissue fresh frozen (FF), formalin- or PAXgene Tissue fixed and paraffin-embedded (FFPE or PFPE), in five different SYBR-Green real time, one step RT-PCR assays. Amplicons of the rat beta-actin gene ranged from 109 to 569 nucleotides (nt).

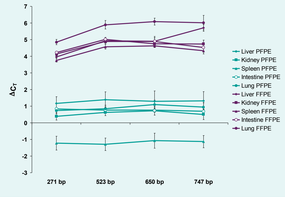
Average delta-C_T values ($\Delta C_T = C_{T[FFPE]} - C_{T[FF]}$ and $\Delta C_T = C_{T[FFPE]} - C_{T[FF]}$) are shown for triplicate extractions amplified in duplicate for each type of tissue and fixation method.

Figure 5: DNA Integrity



Analysis of 300ng DNA in agarose gel electrophoresis (0.8% agarose, TAE buffer). DNA was isolated fresh frozen (FF), formalin- or PAXgene Tissue fixed rat tissue and embedded in paraffin (FFPE or PFPE).

Figure 6: DNA performance in qPCR



Amplification of 10 ng genomic DNA each from rat tissue fresh frozen (FF), formalin- or PAXgene Tissue fixed and paraffin-embedded (FFPE or PFPE), in four different SYBR-Green real time one step PCR assays. Amplicons of the rat beta-actin gene ranged from 271 to 747 nucleotides (nt).

Average delta-C_T values ($\Delta C_T = C_{T[FFPE]} - C_{T[FF]}$ and $\Delta C_T = C_{T[FFPE]} - C_{T[FF]}$) are shown for triplicate extractions amplified in duplicate for each type of tissue and fixation method.

Conclusions

Tissue samples fixed/stabilized with PAXgene Tissue and stored for 3 years at -80°C in PAXgene Tissue Stabilizer:

- **Morphology:** well preserved, comparable to freshly prepared FFPE samples
- **RNA:** RNA integrity scores between 6 to 8
- **DNA:** High molecular weight, in the range of 10 to 23 kb
- **Performance in qRT-PCR:** comparable to RNA from fresh frozen tissue, independent from amplicon length
- **Performance in qPCR:** comparable to DNA from fresh frozen tissue

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