Long-term Storage of Tissue Specimen at -20°C to -80°C with Preservation of Morphology and Nucleic Acids within Frozen Tissue

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Introduction

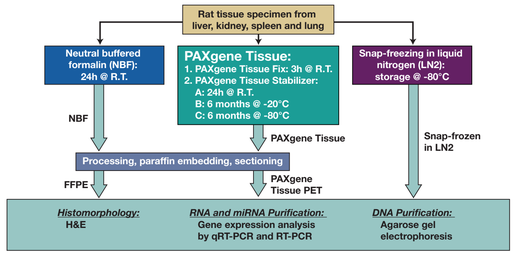
PreAnalytiX has developed a system for preservation of histomorphology and nucleic acids in paraffin embedded tissue (PET) samples. The system is comprised of a collection container for formalin-free fixation and stabilization of tissue specimens plus purification kits for isolation of DNA, RNA or microRNA (miRNA) from PET.

To preserve RNA in tissue for gene expression analysis, snap-freezing in liquid nitrogen (LN2) is the preferred method; but histomorphology in snap-frozen tissue is usually damaged, and sectioning of unfixed, frozen samples is technically demanding, requiring special equipment. The histomorphology of formalin-fixed, paraffin embedded (FFPE) tissue is preserved, but the quality of nucleic acids obtained from FFPE tissue is poor.

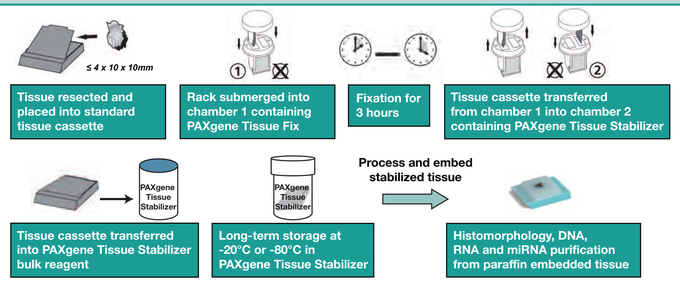
The objectives of this research study were:

1) To investigate whether specimens treated with the PAXgene® Tissue System can be stored in the PAXgene Tissue Stabilizer reagent at -20°C or -80°C without damage to tissue histomorphology, and
2) To investigate whether intact nucleic acids, DNA, RNA and miRNA, can be recovered from PAXgene Tissue treated samples first stored at -20°C or -80°C, and after processing and embedding in paraffin.

Study Design



Fixation/Stabilization in PAXgene Tissue Container and Storage in PAXgene Tissue Stabilizer

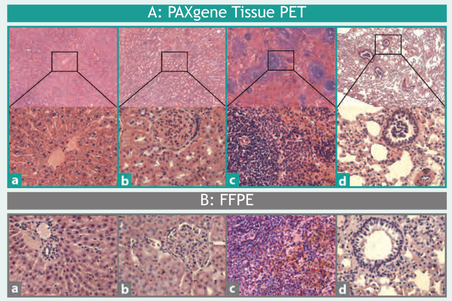


Materials and Methods

NBF	4% neutral buffered formalin
RNA Isolation, FFPE:	RNeasy® FFPE Kit (QIAGEN)
DNA Isolation, FFPE:	QIAamp® DNA FFPE Kit (QIAGEN)
RNA Isolation, PAXgene Tissue PET:	PAXgene Tissue RNA Kit (PreAnalytiX)
miRNA Isolation, PAXgene Tissue PET:	PAXgene Tissue miRNA Kit (PreAnalytiX)
DNA Isolation, PAXgene Tissue PET:	PAXgene Tissue DNA Kit (PreAnalytiX)
RNA Isolation, LN2:	RNeasy® Mini Kit (QIAGEN)
miRNA Isolation, LN2:	miRNeasy® Kit (QIAGEN)
DNA Isolation, LN2:	QIAamp DNA Mini Kit (QIAGEN)
RT-PCR:	QIAGEN® OneStep RT-PCR Kit (QIAGEN)
qRT-PCR:	QuantiTect® Probe RT-PCR Kit (QIAGEN) QuantiTect SYBR® Green RT-PCR Kit (QIAGEN) TaqMan® Gene expression assays (Applied Biosystems)
miRNA qRT-PCR:	TaqMan MicroRNA Reverse Transcription Kit, hsa-miR primer/probe assays (Applied Biosystems)

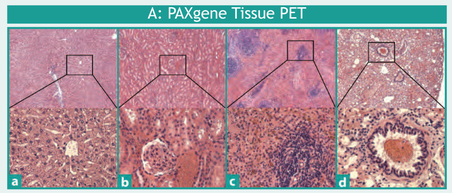
Results

Figure 1: H&E stained sections of PAXgene Tissue PET processed after 6 months in PAXgene Tissue Stabilizer at -20°C and FFPE processed after 24h fixation with NBF



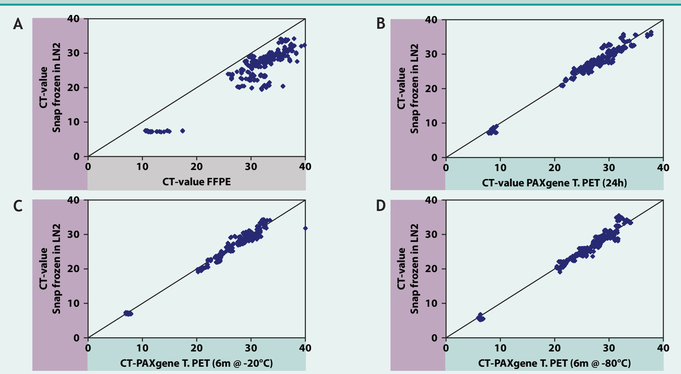
Hematoxylin and eosin (H&E) stained sections from samples of rat liver (a), kidney (b), spleen (c) and lung (d); PAXgene Tissue PET processed after 6 months storage at -20°C in PAXgene Tissue Stabilizer (A) or FFPE, processed after 24h fixation with NBF (B); magnifications x40 and x200.

Figure 2: H&E stained sections of PAXgene Tissue PET processed after 6 months in PAXgene Tissue Stabilizer at -80°C



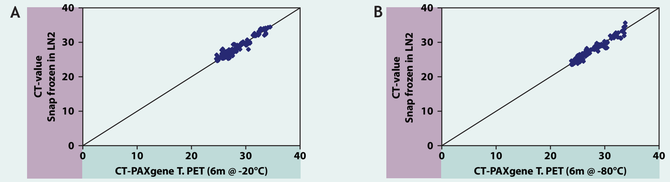
Hematoxylin and eosin (H&E) stained sections from samples of rat liver (a), kidney (b), spleen (c) and lung (d); PAXgene Tissue PET processed after 6 months storage at -20°C in PAXgene Tissue Stabilizer; magnifications 40x and 200x.

Figure 3: Correlation of gene expression levels in snap-frozen tissue, FFPE tissue and PAXgene Tissue PET processed after tissue storage at various conditions



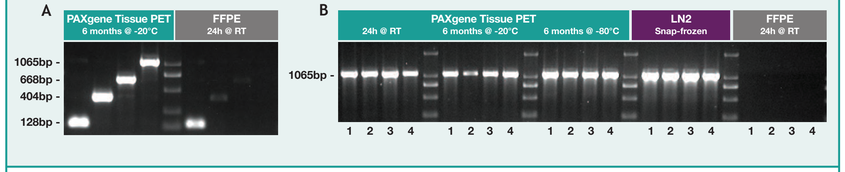
Expression levels of 9 different genes (transferrin receptor, TATA box binding protein, MAD homolog 7, hydroxymethylbilane synthase, hypoxia inducible factor 1, c-fos, c-jun, 18s rRNA and βactin) quantified with SYBR-green or primer/probe quantitative real time RT-PCR. The RNA was isolated from four different rat organs: liver, kidney, spleen and lung. CT-values of RNA from FFPE tissue (A), or PAXgene Tissue PET after storage in PAXgene Tissue Stabilizer for 24h @ RT (B), 6 months @ -20°C (C) and 6 months @ -80°C (D) were plotted in a scatter plot against snap-frozen tissue.

Figure 4: Correlation of miRNA expression levels in snap-frozen tissue and PAXgene Tissue PET processed after tissue storage at various conditions



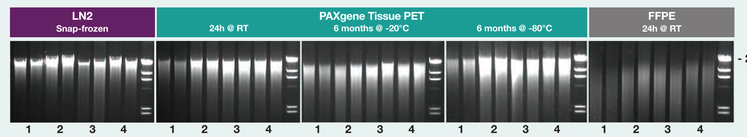
Expression levels of 6 different miRNA genes (miR-103, -192, -16, -30b, -10a and -29a) quantified with primer/probe quantitative real time RT-PCR. The RNA was isolated from four different rat organs: liver, kidney, spleen and lung. CT-values of miRNA genes from PAXgene Tissue PET after storage in PAXgene Tissue Stabilizer for 6 months @ -20°C (A) or 6 months @ -80°C (B) were plotted in a scatter plot against miRNA from snap-frozen tissue.

Figure 5: Endpoint RT-PCR of gene fragments ≤ 1kb with RNA from PAXgene Tissue PET, FFPE or snap-frozen tissue



One step, endpoint RT-PCR of 128, 404, 668 and 1065bp sequences of the rat hypoxanthine phosphoribosyl transferase (HPRT) mRNA with RNA from rat intestine tissue (A) and of the 1065bp sequence only with RNA from different rat organs: liver (1), kidney (2), spleen (3) and lung (4) (B). The RNA was isolated from FFPE or tissue snap-frozen in liquid nitrogen, or from PAXgene Tissue PET after storage in PAXgene Tissue Stabilizer for 24h at RT, 6 months @ -20°C or 6 months @ -80°C prior to processing and paraffin embedding.

Figure 6: Gel electrophoresis of genomic DNA isolated from PAXgene Tissue PET, snap-frozen or FFPE tissue



Agarose gel electrophoresis (0.8% agarose, TAE buffer) of 400ng DNA isolated from duplicate samples of different rat organs: liver (1), kidney (2), spleen (3) and lung (4). The DNA was isolated from tissue snap-frozen in liquid nitrogen, or from PAXgene Tissue PET after storage in PAXgene Tissue Stabilizer for 24h, 6 months @ -20°C or 6 months @ -80°C prior to processing and paraffin embedding or from FFPE tissue.

Conclusion

Preservation of / with	PAXgene Tissue System	Neutral Buffered Formalin	Liquid Nitrogen
Histomorphology	• Comparable to FFPE • Preserved, even after long-term storage in PAXgene Tissue Stabilizer @ -20°C to -80°C • Standardized workflow	• Pre-embedding long-term storage not possible • Under- or over-fixation possible	• Histomorphology compromised due to formation of ice crystals
RNA & miRNA	• Preserved without chemical modification • No significant change in expression level after storage • No RT-PCR inhibition • Intact RNA; fragments > 1kb can be amplified	• Cross-linked, chemically modified • Gene quantification difficult • RT-PCR inhibited • RNA degraded; fragments ≥ 400bp difficult to amplify	• Preserved • Purification technically demanding
DNA	• Preserved without chemical modification • High molecular weight	• Cross-linked, chemically modified • Degraded	• Preserved

Summary

The PAXgene Tissue System enables freezing of tissue specimens in the PAXgene Tissue Stabilizer reagent at -20°C or -80°C for at least 6 months (study ongoing) while preserving histomorphology and molecular content.