New Fixation Technology for Simultaneous Preservation of Morphology and Nucleic Acids in Tissue

[1]Daniel Grölz, [2]Christian Lenz, [2]Nadine Dettmann, [2]Mario Hilker, [2]Evelyn Tränert, [1]Uwe Oelmüller, [1]Lynne Rainen

[1]PreAnalytiX GmbH, Hombrechtikon, Switzerland; [2]QIAGEN GmbH, Hilden, Germany

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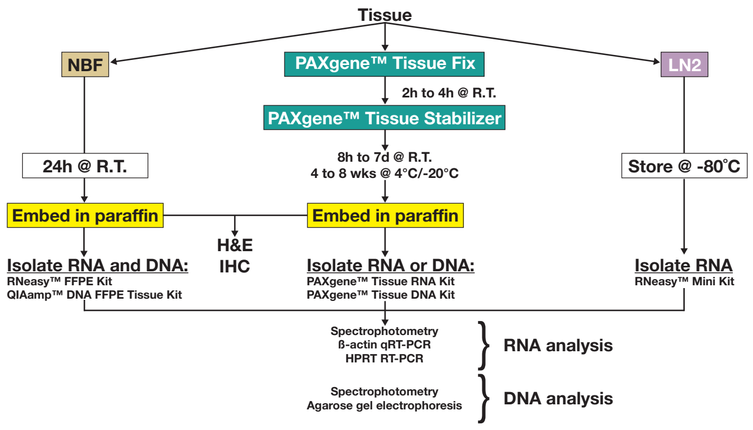
Introduction

Current tissue fixation methods used in traditional histology are of limited use for molecular analysis. Fixatives which contain formaldehyde crosslink biomolecules and destroy or modify nucleic acids and proteins during fixation. Fixation of tissue in liquid nitrogen preserves RNA and DNA but ultimately leads to disruption of morphological structures. We have developed the PAXgene™ Tissue System, which stabilizes nucleic acids, preserves tissue histomorphology, and provides for purification of tissue RNA, micro RNA (miRNA) or DNA.

The PAXgene Tissue System fixes and stabilizes tissue samples with the PAXgene™ Tissue Fix and the PAXgene™ Tissue Stabilizer in a special, dual-chambered container (PAXgene-treated tissue), and the companion PAXgene™ Tissue DNA, RNA or miRNA Kits provide for purification of high quality nucleic acids from PAXgene-treated tissue samples.

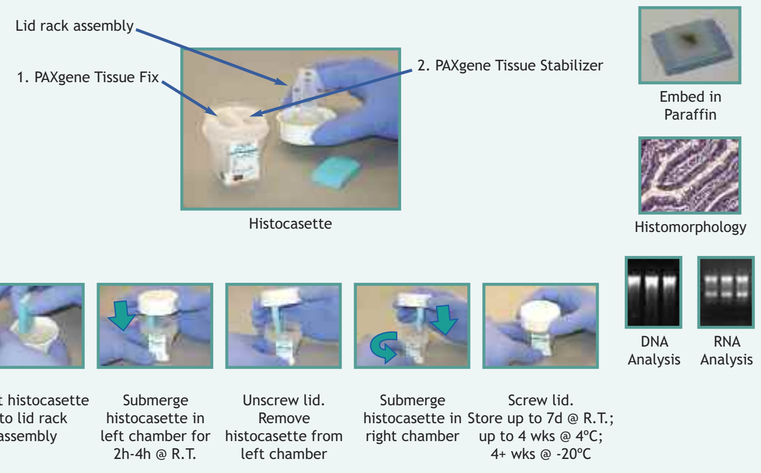
The objective of this study was to evaluate the performance characteristics of the new PAXgene Tissue System as compared to tissue fixed in neutral buffered formalin (NBF) or frozen in liquid nitrogen (LN2). Tissues fixed in either PAXgene or NBF were compared for histomorphology by hematoxylin/eosin (H&E) and immunohistochemistry (IHC) staining intensity. All tissues, PAXgene-treated, NBF-fixed, or frozen in LN2, were compared for RNA and DNA integrity and performance of isolated RNA in RT-PCR assays.

Study Design



Materials and Methods

- Fixation/Stabilization in PAXgene Tissue Container



- RNA Isolation: QIAGEN RNeasy™ FFPE Kit, PAXgene™ Tissue RNA Kit, QIAGEN RNeasy™ Mini Kit
- DNA Isolation: QIAGEN QIAamp™ DNA FFPE Tissue Kit, PAXgene™ Tissue DNA Kit
- Real time RT-PCR: QIAGEN QuantiTect™ Probe RT-PCR Kit
- RT-PCR: QIAGEN OneStep™ RT-PCR Kit

Results

Figure 1: Histomorphology of PAXgene-treated and NBF-fixed samples

Hematoxylin and eosin (H&E) stained sections (200x original magnification) from mirrored samples of rat liver (a), kidney (b), spleen (c) and intestine (d) treated with PAXgene (A) or fixed in neutral buffered formalin (NBF) (B).

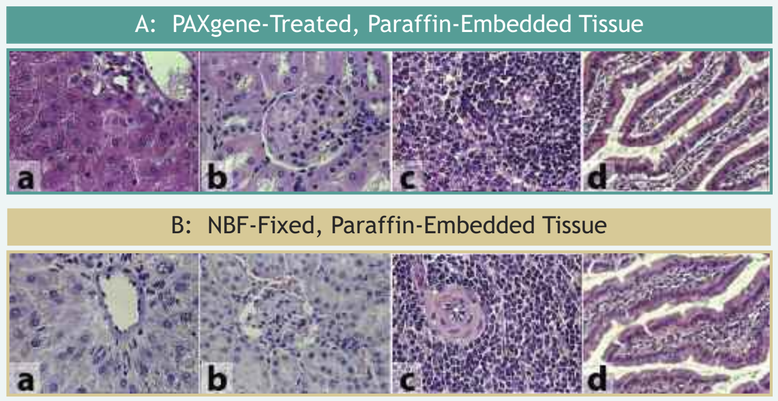


Figure 2: Histomorphology of samples stored in PAXgene Tissue Stabilizer for up to 8 weeks at different temperatures

Hematoxylin and eosin-stained sections (200x original magnification) from rat liver (a), kidney (b), spleen (c) and intestine (d) fixed with PAXgene Tissue Fix for 4 hours, transferred into PAXgene Tissue Stabilizer and stored for 7 days at 22°C (A), 8 weeks at 4°C (B) or 8 weeks at -20°C (C) before processing.

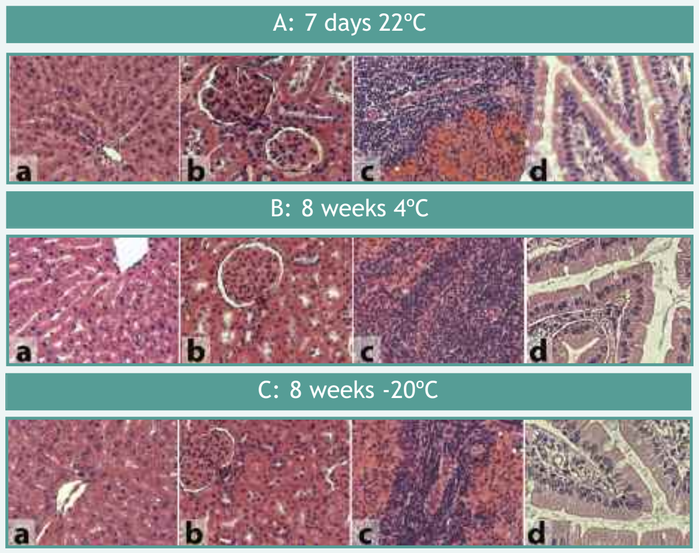


Figure 3: Ki-67 IHC assay of PAXgene-treated and NBF-fixed samples

Sections (200x original magnification) of rat liver (a), kidney (b) and intestine (c) treated with PAXgene (A) or fixed in NBF (B). Demonstration of the Ki-67 antigen in a labeled streptavidin-biotin assay with MIB-5 antibody, visualization with DAB+, counterstain with hematoxylin.

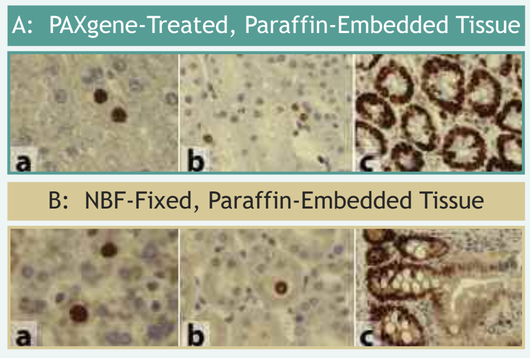
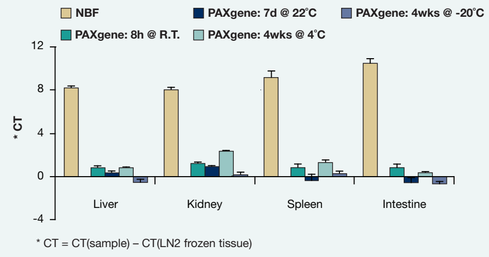


Figure 4: Gene expression levels of PAXgene-treated and NBF-fixed tissue as compared to LN2 frozen tissue controls

Rat tissue samples were fixed either in NBF for 24h at room temperature or treated with PAXgene as indicated in the figure below. After processing, RNA from PAXgene-treated or NBF-fixed, paraffin-embedded tissues was analyzed by quantitative ß-actin real time RT-PCR and compared to assay results from tissues frozen in LN2.



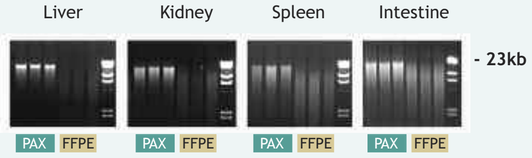
* CT = CT(sample) – CT(LN2 frozen tissue)

Figure 5: One-step RT-PCR of RNA isolated from PAXgene-treated, NBF-fixed, or frozen tissues

One-step RT-PCR of a 1065bp sequence of the rat hypoxanthine phosphoribosyl transferase (HPRT) mRNA. RNA was isolated from duplicate LN2 frozen (LN2), NBF-fixed, paraffin-embedded (FFPE), or PAXgene-treated, paraffin-embedded (PAX) tissue samples.

Figure 6: Agarose gel of DNA purified from PAXgene-treated or NBF-fixed tissue

Agarose gel electrophoresis (0.8% agarose, TAE buffer) of 300ng DNA isolated from triplicate rat NBF-fixed, paraffin-embedded (FFPE) or PAXgene-treated, paraffin-embedded (PAX) tissue samples.



Conclusions

- Histomorphology of PAXgene-treated, paraffin-embedded tissue samples is comparable to that of NBF-fixed, paraffin-embedded tissue samples.
- IHC staining intensity is comparable between PAXgene-treated, paraffin-embedded and NBF-fixed, paraffin-embedded tissue samples.
- High molecular weight RNA and DNA can be isolated from sections of PAXgene-treated, paraffin-embedded tissue using the PAXgene Tissue RNA and DNA kits.
- Tissue fixed in PAXgene Tissue Fix can be stored in PAXgene Tissue Stablizer at 18 to 22°C for up to 7 days and at 2 to 8°C or -20°C for at least 4 weeks before processing without affecting histomorphology, DNA or RNA yield or integrity, or performance in downstream research applications such as RT-PCR or qRT-PCR.
- All RNA samples from sections of PAXgene-treated, fixed, paraffin-embedded tissue show similar performance in real time qRT-PCR compared to RNA isolated from samples quick-frozen in liquid nitrogen.
- All RNA samples from sections of PAXgene-treated, fixed, paraffin-embedded tissue exhibited successful RT-PCR amplification of a 1065bp mRNA sequence.

Summary

In this study we have shown that the PAXgene™ Tissue System stabilizes molecular content while preserving histomorphology in tissue, thus enabling both molecular and traditional pathology testing from the same specimen.