Preservation of Histomorphology and Nucleic Acids in Human Breast Tumor Tissue with the New PAXgene® Tissue System – A Study with Comparison to Formalin Fixation

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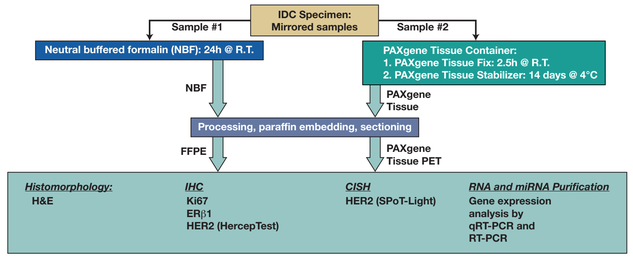
Introduction

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.

The objective of this research study was to investigate whether the PAXgene Tissue System can be used with routine H&E, IHC and HER2 expression analysis performed on breast tumor tissue. Additionally, we sought to compare molecular analysis of RNA and miRNA isolated from PAXgene Tissue PET to that of formalin-fixed paraffin embedded (FFPE) tissue.

In this case study, mirrored samples of a human infiltrating ductal carcinoma (IDC) of the breast were fixed with neutral buffered formalin (NBF) or treated with PAXgene Tissue Container Fix and Stabilizer. After processing and paraffin embedding, samples were analyzed by a variety of histopathological and molecular methods and the results were compared.

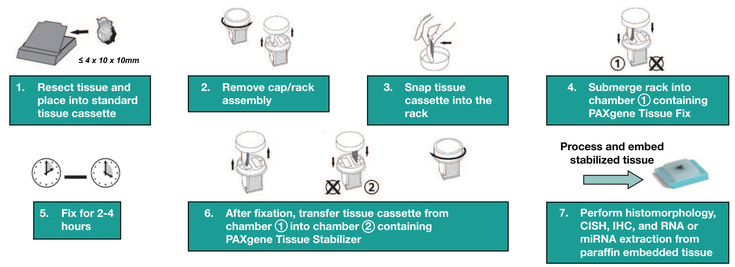
Study Design



Materials and Methods

NBF:	4% neutral buffered formalin
RNA Isolation, FFPE:	RNeasy® FFPE Kit (QIAGEN)
RNA Isolation, PAXgene Tissue PET:	PAXgene® Tissue RNA Kit (PreAnalytiX)
miRNA Isolation, FFPE :	miRNeasy FFPE Kit (QIAGEN)
miRNA Isolation, PAXgene PET:	PAXgene® Tissue miRNA Kit (PreAnalytiX)
RT-PCR:	QIAGEN® OneStep RT-PCR Kit (QIAGEN)
qRT-PCR:	QuantiTect™ Probe RT-PCR Kit (QIAGEN)
miRNA qRT-PCR:	TaqMan® MicroRNA Reverse Transcription Kit, hsa-miR primer/probe assays (Applied Biosystems)
HER2-IHC:	HercepTest™ (Dako)
HER2 CISH:	SPoT-Light™ HER2 CISH Kit (Invitrogen)

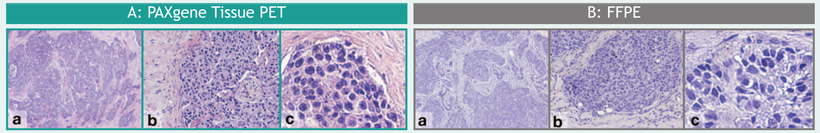
Fixation/Stabilization in PAXgene Tissue Container



The PAXgene Tissue System is 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 disease.

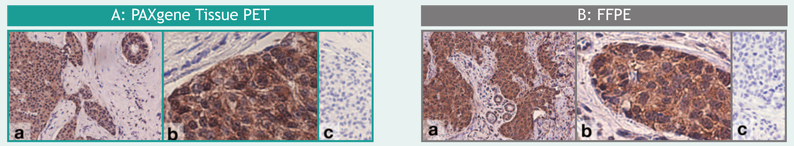
Results

Figure 1: H&E stained sections of PAXgene Tissue PET and FFPE Human Breast Tumor Tissue



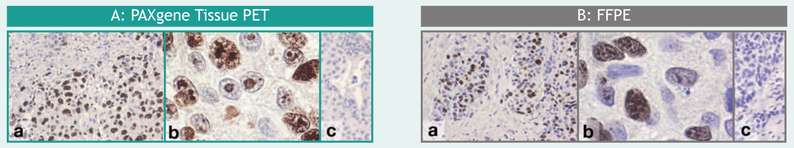
Hematoxylin and eosin (H&E) stained sections from mirrored samples of IDC of the human breast; PAXgene Tissue PET(A) or FFPE (B), 40x (a), 100x (b), 400x (c).

Figure 2: Immunohistochemical analysis of estrogen receptor ß1 (ERß1) expression with PAXgene Tissue PET and FFPE human breast tumor tissue



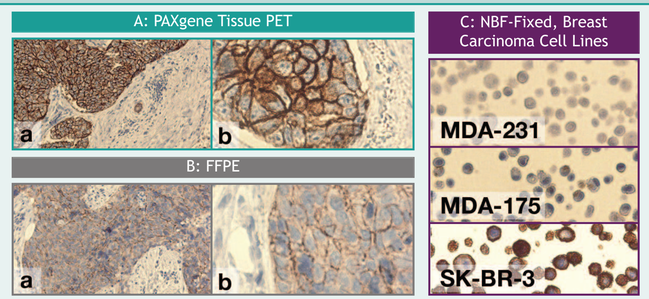
IHC (immunohistochemistry) staining of ERß1 antigen in a labeled streptavidin-biotin assay counterstained with hematoxylin. Mirrored samples of IDC of the human breast; PAXgene Tissue PET (A) or FFPE (B), 100x (a), 400x (b), neg. controls with unspecific IgG2 200x (c). For the PAXgene Tissue treated sample antigen retrieval was not needed and was omitted.

Figure 3: Immunohistochemical analysis of Ki67 antigen expression with PAXgene Tissue PET and FFPE human breast tumor tissue



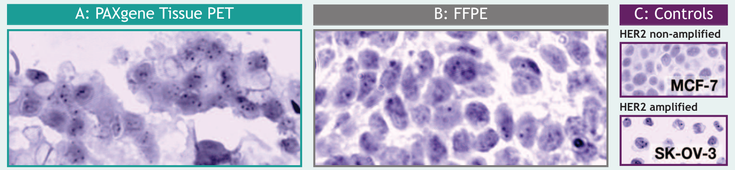
IHC staining of Ki67 antigen with MIB-1 antibody in a labeled streptavidin-biotin assay counterstained with hematoxylin. Mirrored samples of IDC of the human breast; PAXgene Tissue PET (A) or FFPE (B), 200x (a), 630x (b), neg. controls with non-specific IgG1 200x (c).

Figure 4: HercepTest IHC assay of PAXgene Tissue PET and FFPE human breast tumor tissue



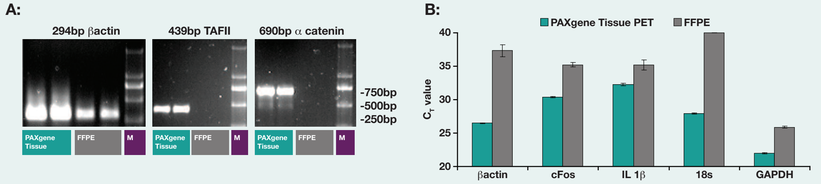
HercepTest (Dako) for determination of HER2 (human epidermal growth factor receptor 2) protein overexpression. Assay performed according to manufacturer's instructions with sections from mirrored samples of IDC of the human breast; PAXgene Tissue PET (A) FFPE (B), 100x (a), 400x (b) and with three formalin-fixed, breast carcinoma cell lines (C) representing different levels of HER2 protein expression MDA-231 (0), MDA-175 (1+) and SK-BR-3 (3+). For the PAXgene Tissue PET sample, antigen retrieval was not needed and was omitted.

Figure 5: HER2 chromogenic in situ hybridization (CISH) assay of PAXgene Tissue PET and FFPE human breast tumor tissue



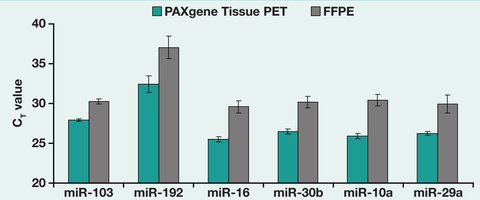
The SPoT-Light HER2 CISH Kit was used for confirmation of HER2 protein overexpression. Assay was performed according to manufacturer's instructions with mirrored samples of IDC of the human breast, treated with PAXgene Tissue PET (A) or FFPE tissue (B), and with formalin-fixed control cell-lines HER2 non-amplified MCF-7 and HER2 amplified SK-OV-3 (C); all pictures shown with 600x magnification. PAXgene Tissue PET sample was prepared for hybridization without heat pretreatment step and enzyme digestion was not needed and was omitted.

Figure 6: Gene expression analysis by endpoint or quantitative real time RT-PCR with RNA isolated from PAXgene Tissue PET and FFPE tissue



One step, endpoint RT-PCR (A) of human genes of increasing amplicon length (294bp ßactin, 439 TAFII and 690bp α catenin, with marker [M]) and quantitative real time RT-PCR (B) of five different housekeeping or single copy genes of RNA (10ng each), isolated from mirrored samples of IDC of the human breast treated with PAXgene Tissue or fixed with NBF.

Figure 7: Gene expression analysis of six different miRNA genes by quantitative real time RT-PCR with RNA isolated from PAXgene Tissue PET and FFPE



Quantitative real time RT-PCR with primer/probe assays of six different miRNA genes (miR-103, -192, -16, -30b, -10a and -29a) with 5ng RNA isolated from mirrored samples of IDC of the human breast treated with PAXgene Tissue or fixed with NBF.

Conclusion

In conclusion, the test results yielded the following comparison between PAXgene Tissue PET and FFPE breast tumor (IDC) tissue:

Test	Features & Advantages of PAXgene Tissue System	Differences from FFPE
H&E stain	• Histomorphology equivalent to FFPE • Chromatin structure and nuclear details were clearer in the PAXgene Tissue PET	• PAXgene Tissue PET slightly more eosinophilic
IHC	• Staining intensity was higher for HER2 in the HercepTest • ERß1 staining intensity easily increased by altering antigen-specific antibody concentration • Antigen retrieval not necessary for HER2 and ERß1	• Staining intensity for ERß1 higher in FFPE
CISH	• CISH analysis with the SPoT-Light HER2 CISH Kit confirmed amplification of HER2 • Heat pretreatment step not necessary for HER2	
RT-PCR qRT-PCR	• Assay results for RNA and RT-PCR better and more reliable than those for FFPE	

Summary

We have demonstrated that the PAXgene Tissue System can be used with routine H&E, IHC and HER2 expression analysis on breast tumor tissue. Additionally, the quality of RNA and miRNA isolated from PAXgene Tissue PET is better than that of FFPE tissue as shown by molecular analysis.