Can we preserve morphology and nucleic acid integrity for molecular pathology?

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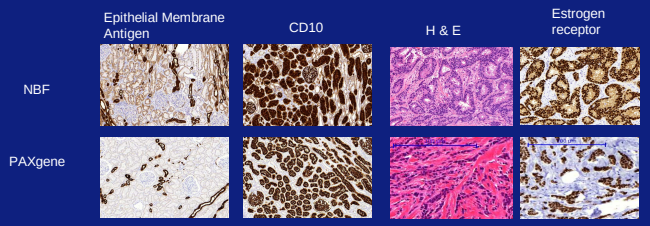
INTRODUCTION

Short sequences of DNA and RNA can be retrieved from tissue fixed in neutral-buffered formalin (NBF). However, molecular diagnostic and research techniques increasingly require material of a quality higher than that extractable from NBF. PAXgene is a new, commercially available, alcohol-based tissue fixative, produced by Qiagen as a molecular biology-friendly alternative to NBF. The PAXgene system consists of easy to use, dual-cavity tissue containers pre-filled with the fixative and the stabilizing solutions required for tissue preservation.

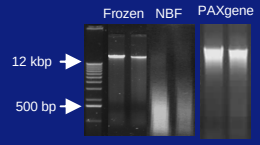
Tissue can remain in the stabilizing solution for several days at room temperature, during which time, for example, the container can be mailed to a central processing centre for paraffin embedding and histology. The aim of this study was to evaluate PAXgene by comparing it with NBF in terms of preserving morphological detail and maintaining the integrity of extractable nucleic acid (DNA and RNA). Snap frozen samples were included in the molecular biology work as the gold standard.

RESULTS 1: MORPHOLOGY



NBF-fixation produced little shrinkage and loss of preservation at both the nuclear and cytoplasmic level and preserved morphology well. PAXgene sections were similar in quality to NBF, but were more eosinophllic when stained with H & E.

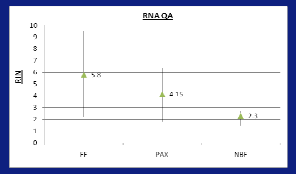
RESULTS 2: DNA



Only degraded DNA was obtainable from NBF (<1 kbp in size) but PAXgene and frozen samples generated DNA > 15 kbp in length. Most samples, regardless of fixative, produced amplimers of 400-600 bp following multiplex PCR.

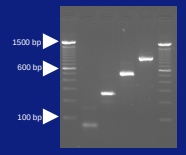
RESULTS 3: RNA

AGILENT BIOANALYSER



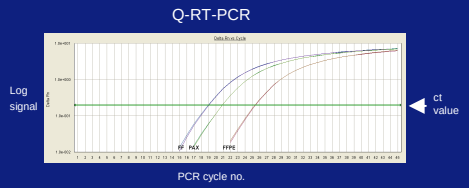
RNA degradation is measured by RIN numbers, ranging from 1 (completely degraded) to 10 (completely intact). Median RINs were 5.8 (range 2.2-9.5) for frozen, 4.15 (range 1.8-6.4) for PAXgene and 2.3 (range 1.5-2.7) for NBF.

SIZE-RT-PCR



Amplicons of 65, 256, 534 or 942 bp of hydroxymethylbilane synthase were sought by RT-PCR. 942bp amplicons were obtained from all frozen and PAXgene samples, but only the 65 bp amplicon was obtainable from NBF samples.

Q-RT-PCR



Four matched samples were subjected to Q-RT-PCR for β-actin, GFRA1 (glial cell line derived neurotrophic factor family receptor alpha 1), GDNF (glial cell derived neurotrophic factor) and Ret oncogene. The cycle threshold (ct) value (green) inversely represents the amount of RNA in the sample. There was a similar plot for all four tissue samples and for all transcripts (where gene expression occurs). The ct values from NBF were consistently and markedly higher than those from frozen and PAXgene-fixed tissue.

METHODS

Tissue samples from the same operative specimen were snap-frozen or fixed, either in NBF or PAXgene. Morphological detail was assessed in 4 µm sections stained with either H & E or antibodies appropriate to the tissue type. DNA and RNA were extracted using Qiagen kits then quality assessed using nanodrop spectrophotometry followed by gel electrophoresis and multiplex PCR (for DNA) and the Agilent Bioanalyser, RT-PCR for different sized amplimers and Q-RT-PCR (for RNA).

CONCLUSIONS

The necessity to preserve morphology for diagnosis using NBF compromises the integrity of nucleic acid, thereby diminishing the value of the tissue for molecular diagnostics and research. Our study demonstrates that the PAXgene preservation system is a significant advance in that it offers greatly improved preservation of nucleic acid without compromising the histological detail associated with NBF. However, RNA quality still falls short of that obtainable from fresh-frozen tissue (the current gold standard). Therefore, we think it is unlikely that PAXgene will replace the necessity for frozen tissue for some applications, such as gene expression arrays. Nevertheless, we are encouraged by these results so are currently investigating whether DNA extracted from PAXgene-fixed tissue can be used in methylation arrays, whether improved assessment of copy number alteration by array CGH compared to NBF can be seen and how amenable PAXgene-fixed tissue is to proteomic analysis.

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