Implementation of formalin-free PAXGene tissue fixative into routine use: evaluation of H&E morphology, IHC and FISH

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INTRODUCTION

- Formalin is the standard method of fixation; immunohistochemistry (IHC) and FISH protocols are optimised for this material
- Formalin is damaging to nucleic acids, precluding application of molecular techniques
- PAXGene fixative is designed to preserve nucleic acids, yielding higher quality DNA/RNA
- This study aims to evaluate the performance of PAXGene-fixed paraffin-embedded (PFPE) tissue in routine histological techniques.

METHODS

•75 paired tumour samples were fixed in Formalin and PAXgene, processed overnight and embedded in paraffin
•H&E slides underwent blinded morphological assessment
•IHC was undertaken on 45 cases and reviewed to identify antibodies performing inadequately and requiring optimization with PFPE tissue
•FISH, using EGFR and CDKN2A probes, was performed on 20 paired cases using an optimised protocol (consisting of a 24 hour step in formalin)

Tissue Type	Number
Prostate	43
Colorectal	25
Oesophagus	3
Lymph Node	2
Lung	1
Ovary	1

Table 1. Sample collection by tissue type.

MORPHOLOGICAL ASSESSMENT

	Cytoplasm	Cell Membrane	Nuclear	Overall
FFPE	3.4857	3.4714	3.5286	10.486
PFPE	3.5429	3.6286	3.7857	10.957
P-value	0.25	0.04	0.001	0.02

Table 2. Morphological assessment of FFPE vs PFPE H&Es. Overall scores were significantly higher in PFPE tissues, as well as cell membrane and nuclear staining. No significant difference was found in cytoplasmic staining. Overall score out of 12.

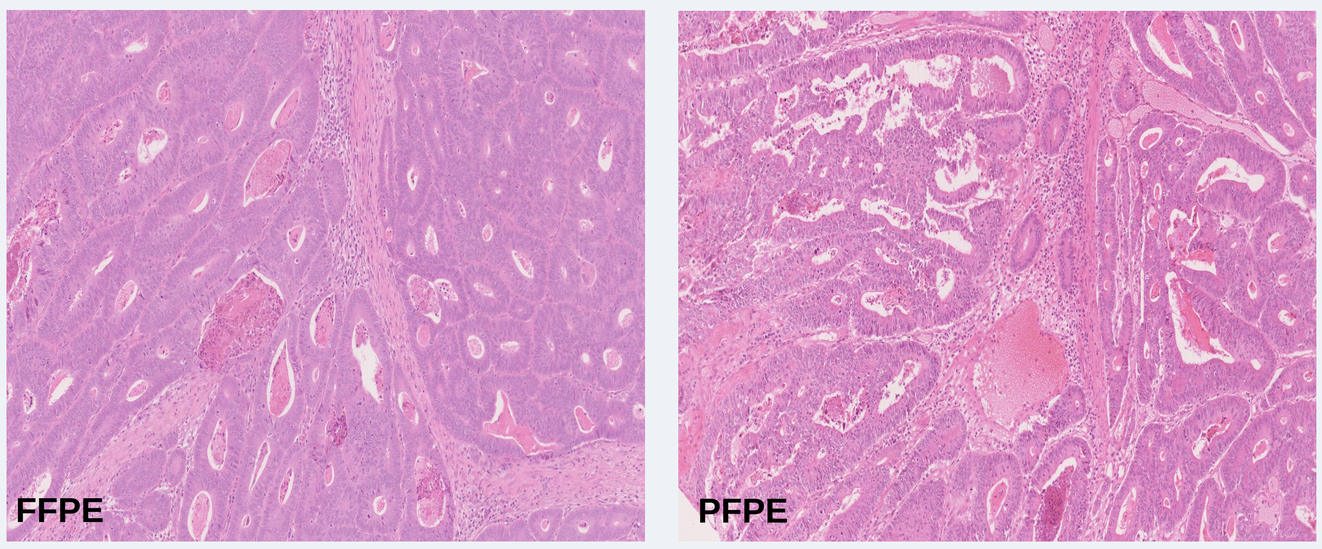

Figure 1. H&E sections from a colorectal case,

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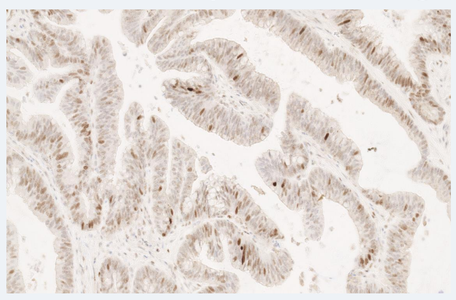
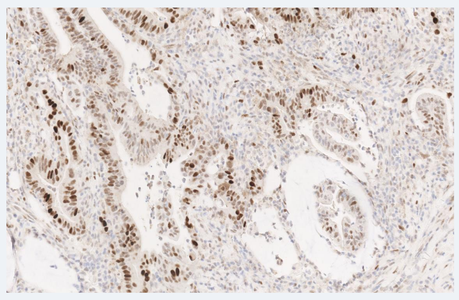
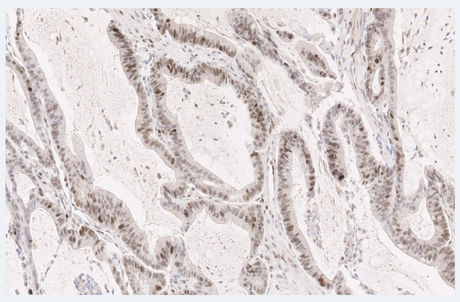
p53 and EGFR immunoreactivity in PFPE samples was variable and suboptimal for diagnosis. p53 IHC was subsequently repeated with an optimized protocol (1 hour in formalin after dewaxing), and deemed adequate for diagnosis.



PFPE: standard method



FFPE: standard method



PFPE: optimised method

Figure 2. p53 staining in a CRC case.

Tissue	Antibodies
Prostate (n=14)	P63, 34BE12, CK5,, p63/racemase (AMACR)
Colorectal (n=25)	MLH1, MSH2, PMS2, MSH6, p53, Her2
Lymph Node (n=2)	CD3, CD4, CD20, MIB-1
Oesophagus (n=3)	P53, Her2
Lung (n=1)	TTF1,CK7, ALK1

Table 5. Antibodies validated for each tissue. EGFR and CDKN2A staining was also performed

Additionally CRC cases were interpreted by a pathologist for gain/loss of MMR proteins and Her2. Interpretations were concordant between FFPE and PFPE sections for every case.

FISH

All cases were deemed adequate for diagnosis. A significant difference was observed in the strength of the signal ($p<0.05$); 36/40 FFPE cases had a “very strong” signal compared to 12/40 PFPE case. All remaining cases had a “strong” signal.

	Nuclear/DAPI counterstaining	Background	Intensity of CEP	Intensity of Target gene
FFPE	2	0.075	3.825	3.825
PFPE	2	0.075	3.250	3.225

Table 4. Average scores of FISH Assessment for EGFR and CDKN2A. Counterstaining scored 0 (absent), 1 (weak), 2 (adequate). Background scored 0 (absent), 1 (weak), 2 (high). Intensity scored 0 (absent), 1 (very weak), 2 (weak), 3 (strong), 4 (very strong). No significant differences were observed.

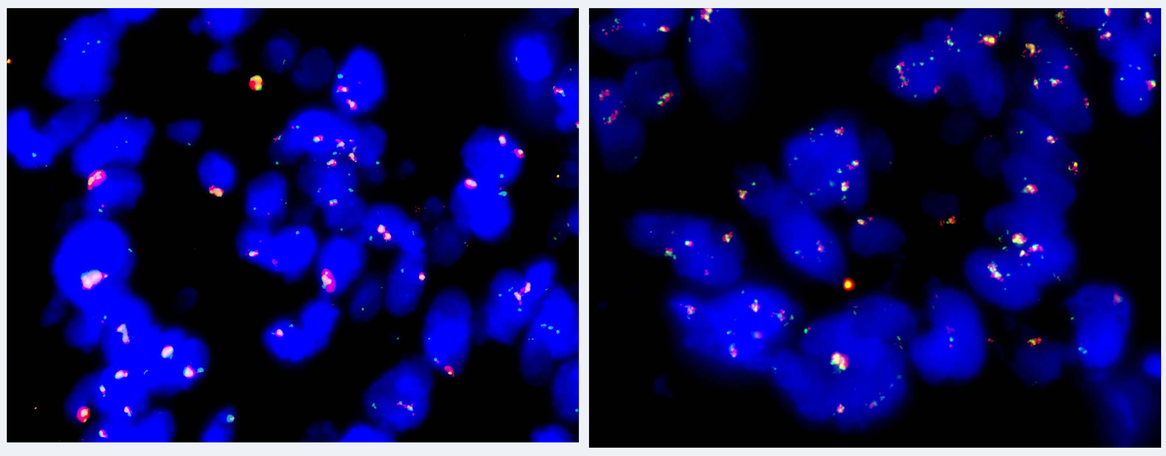


Figure 3. FISH signals from EGFR probe. Left PFPE, right FFPE. EGFR (red), CEP (green)

CONCLUSIONS

PFPE tissue performed favorably to FFPE tissue in blinded scoring of morphology. IHC was successful with the exception of p53 and EGFR where immunoreactivity was variable. Protocol optimization improved p53 staining. FISH performance for both fixation methods was comparable, following optimisation of the protocol.

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