NIHR Cambridge Biomedical Research Centre

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Paxgene; A beneficial formalin alternative to study lung cancer

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Background The STRATfix project is an InnovateUK funded project between University College London, seven NHS Trusts and Qiagen – a leading manufacturer of molecular diagnostic reagents.

Most diagnostic pathology laboratories rely on formalin for the fixation of tissue samples sent for histopathological examination.

Whilst adequate for most existing pathological tests, formalin fixation is detrimental to DNA/RNA quality and carries with it significant health and safety considerations.

Next generation sequencing (NGS) of tumour material from lung cancer patients to identify actionable mutations is emerging as a key strategy in the treatment of lung cancer and is highly dependent on DNA/RNA quality.

Here we compare PAXgene fixation to formalin fixation in:-

1. Block-sized pieces of lung parenchyma
2. Block sized pieces of lung tumour
3. Tenmo needle core biopsies of lung tumour
4. Fine needle aspirates (FNA) of tumour

Methods

Duplicate samples of lung tumour, background lung parenchyma, Tenmo needle core biopsies and FNA tumour samples (to mimic the Endrobronchical Ultrasound-guided (EBUS) FNA samples widely used in the diagnosis and staging of lung cancer) were collected from n=17 informed and consenting individuals with either lung adenocarcinoma or squamous cell carcinoma undergoing surgery at Papworth Hospital NHS Foundation Trust

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Samples fixed in **PAXgene tissue, or PAXgene FNA fixative (for EBUS FNA)** for 12-24 hours, transferred to PAXgene tissue stabiliser and placed into freezer until formalin free processing

Replicate samples fixed in **formalin or cytolyt (for EBUS FNA)** for 12-24 hours and processed using formalin-based processing

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Blocks embedded in paraffin-wax and slides produced following standard laboratory practices

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Haematoxylin and Eosin staining performed and slides blind-scored to evaluate quality of nuclear staining (score 0-4), cytoplasmic staining (score 0-4) and cell membranes (score 0-4) as described by Craft *et al*., 2014

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Immunohistochemistry for Cytokeratin (CK) 7, CK5/6, MNF116, TTF-1, P63 and Ki67 was performed

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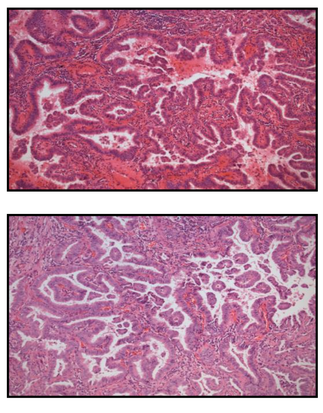
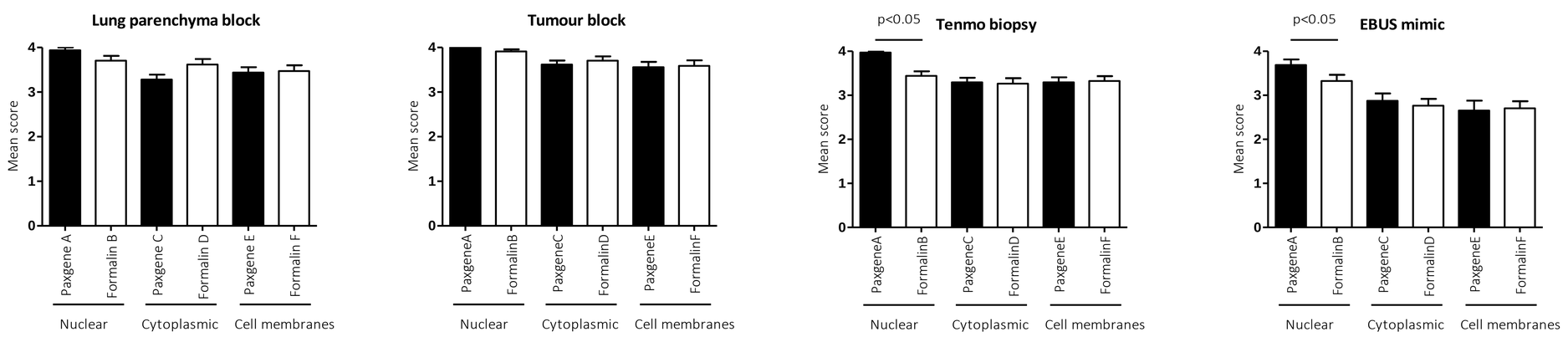
n=5 additional slides from each block cut and referred to QUB for analysis of DNA quality. Now currently pending NGS using the Ion Torrent Ampliseq 50 gene Cancer panel

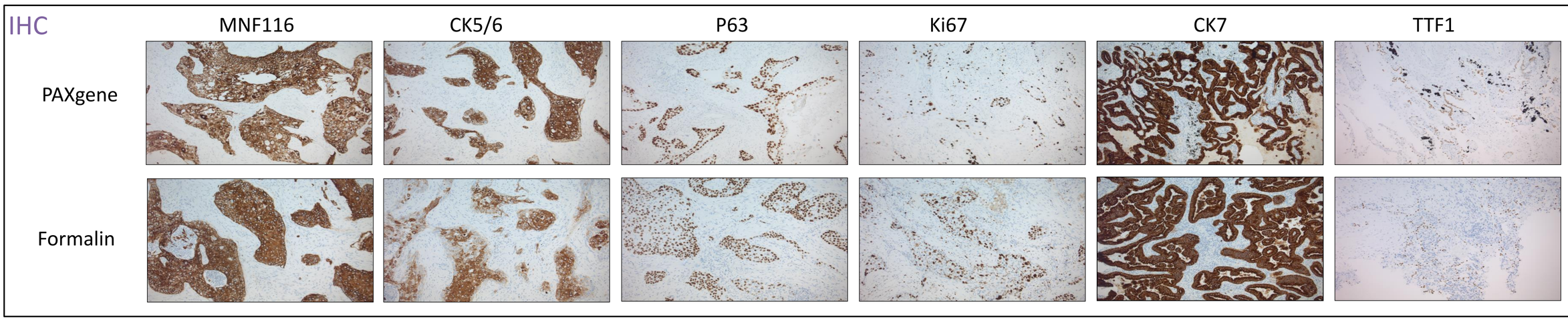
Results

Histology

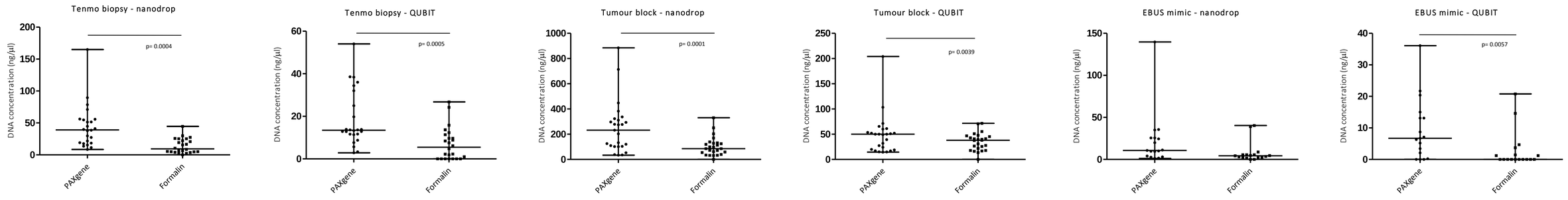




IHC



DNA quality metrics (median value +/- range of data)



Conclusions: PAXgene is comparable to formalin for histopathological study and in some sample types preferential for demonstration of nuclear detail. Equivalent immunohistochemical findings were observed in PAXgene and formalin-fixed samples. Crucially, within the context of preserving DNA/RNA quality and integrity, key for the molecular study of lung cancer driver mutations, superior results were observed in PAXgene fixed samples compared to formalin.

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