Improving Cancer Diagnostics: Investigation of a Novel Fixative on Cell Morphology and DNA preservation in Cytology Samples.

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Introduction

As cancer diagnostics have evolved, minimally invasive samples submitted to cellular pathology are increasingly used to perform molecular testing to obtain diagnostic, prognostic and predictive information about individual tumours that helps to inform personalised and targeted treatment options. Successful testing requires good preservation of proteins and nucleic acids.

The traditional formalin fixative is known to cause degradation of nucleic acids and proteins and has now been classed as a carcinogen. The aim of this study was to demonstrate that a new alcohol based fixative, FNA FIX (FF) could provide improved preservation of DNA and comparable morphology to the routinely used Cytorich Red (CR) fixative in cell blocks made from cytology specimens.

Methods

1. Collect samples of lung FNA and fluids. Divide samples and fix with FNA FIX and Cytorich Red.
2. Process samples to Cellient cells. Cut sections and stain with Haematoxylin and eosin (H&E).
3. Pathologists compare morphology using a scoring system.
4. Extract DNA using PAXgene tissue DNA kit for FNA FIX or QI Amp FFPE tissue kit for Cytorich Red.
6. PCR amplification using the BIOMED-2 primer mix.
7. Fragmentation analysis via capillary electrophoresis.

Results

Morphology – some differences in staining appearance between the fixatives were observed. Only membrane staining was significantly different on Mann Whitney U test but all scored at least 3 out of 4 and were therefore suitable for diagnosis.

DNA purity & quantity – student’s t test found no difference in purity, total DNA or double stranded DNA quantity between fixatives. There was a significantly increased amount of double stranded DNA in fluids compared to FNA samples regardless of fixative.

Fragmentation analysis – 95% cases had identical peaks in the two fixative groups and 1 case had a maximum of 400bp fragment in the CR group and only a 300bp fragment in the FF group. The maximum fragment length in any sample was 400bp and the minimum was 300bp.

Discussion

If FNA FIX were to be implemented into the laboratory it would have to provide comparable morphology to Cytorich Red on H&E stained sections. In this study the morphology and staining was comparable and would be suitable for diagnosis. FF sections had increased eosinophilia which may enhance contrast and slightly improved membrane staining but the red blood cell lysis may be problematic for lymphoid malignancies where they are used as a size reference.

From statistical analysis there seems to be no advantage to using FNA FIX to preserve DNA. Maximum fragments lengths were shorter than those found in other studies looking at cytological fixatives so further investigation is needed to determine whether any methodology or pre-analytical factors other than fixative choice affected this.

Double stranded DNA content may have been higher in fluids compared to FNA because fluids often provide a more cellular starting material. Fluids contain more supernatant, which has been found to contain high levels of DNA.

Future investigations should include immunohistochemistry; an integral part of cancer diagnostics in histopathology and RNA assessment which is becoming increasingly important with emerging next-generation technologies and gene expression studies. Both have been shown to be affected by choice of fixative.

Sub-optimal preservation of DNA for molecular tests may contribute to inaccurate results, potentially denying a patient the right to targeted therapy and new products should only be implemented if they offer an improvement to current practice. Based on the results of this study it is not possible to recommend the implementation of FNA FIX into the laboratory workflow in its current form.

References

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