

# A formalin-free fixative, that provides superior preservation for molecular techniques

## Introduction

The aim of fixation is the preservation of cells and tissue constituents in as life like a form as possible. Despite the search for many years, using numerous combinations of fixing agents and additives, the 'ideal' fixative has alluded histologists.

Diagnostically, the most important feature has always been tissue architecture. More recently, over the last three decades the preservation of proteins within tissues has become more vital, with the evolution of immunohistochemical techniques. It is therefore not surprising that the fixative of choice for routine histological diagnosis, has remained formaldehyde-based. These not only provide excellent morphological preservation, but also protein preservation. The fact that proteins, or more specifically, the antigenic sites that are required to be demonstrated by immunohistochemical techniques become 'masked' due to chemical interactions provided a small set back. Formalin fixation is a reversible reaction, so with the every increasing importance of antibodies as diagnostic tools ways to reverse the masking of antigenic sites were developed.

Now however, we are seeing a new development in routine laboratory practise, the need to perform essential molecular diagnostic and prognostic techniques. The features required for the 'ideal' fixative have now shifted. Formaldehyde-based fixatives are poor preservatives of DNA and RNA. Whilst it could be argued alcohol based fixatives are good for this, they result in poor morphology and to a certain degree tissue antigenicity which are still essential diagnostic criteria.

In this study we evaluate a novel formalin-free fixative, FineFIX, which proposes to offer a combination of good morphological preservation along with preservation of tissues in such a way as to allow essential molecular profiling techniques.

Figure 1: Liver

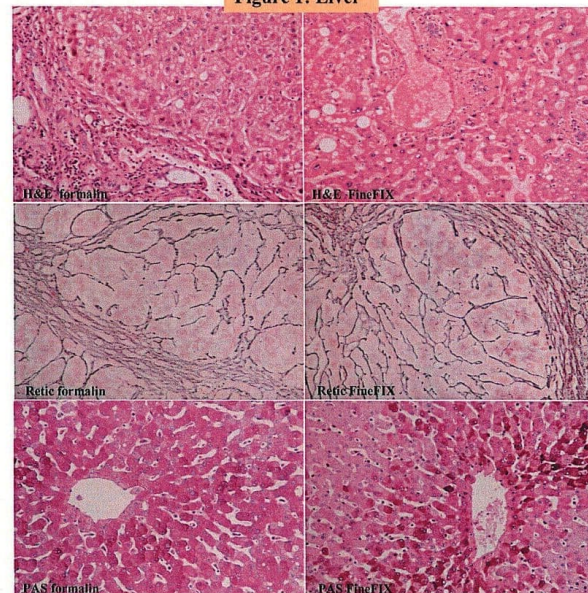


Figure 2: Results from DNA extraction

DNA was extracted from the 4 GIST specimens and used to identify GAPDH, p63, 5 exons from KIT and 3 from PDGFR $\alpha$ .

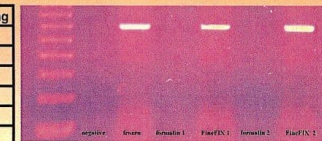
Below are the resultant ethidium bromide gels from 2 of the GIST tumours analysed



Figure 3: Results from RNA extraction

The table below gives the values obtained for RNA integrity and quantity obtained from 8 samples, and the resultant ethidium bromide gel demonstrating the housekeeping gene GAPDH

Specimen	260/280	Quantity ng/mg
frozen liver	2.03	122.3
FineFIX liver	2.04	278
formalin liver	0.9	1.65
frozen GIST 1	2.06	102.8
FineFIX GIST 1	2.05	116.1
formalin GIST 1	1.1	8.5
FineFIX GIST 2	2.04	106.4
formalin GIST 2	1.33	11.6



## Materials and Methods

FineFIX is a water based additive which comes as a concentrate (Sugipath, UK). 3 parts absolute ethanol is added to 1 part FineFIX for use. Specimens were received fresh from theatre and 5 samples were taken from each: 1 snap frozen in liquid nitrogen, 3 placed into FineFIX and 1 into formalin. The snap frozen sample along with 1 of the fine fix samples were placed into a -80° freezer until required. The 3 remaining samples were routinely processed through to paraffin wax, with 1 FineFIX sample receiving a post fixation in formalin on the processor and the other going straight into alcohol. Specimens included 3 livers, 4 gastrointestinal tumours (GIST), 1 oesophageal tumour, 1 colorectal tumour and 1 lymphoma of the thyroid.

4µm sections were cut and placed onto glass slides (coated slides for immunostaining). A hematoxylin and eosin (H&E) were performed on all samples. Standard histochemical stains were performed on the liver samples. A range of antibodies were used on all samples, including CD117 on the GIST and a lymphoma panel on the thyroid. All sections were reviewed by 2 independent assessors.

DNA extraction was performed on paraffin processed tissue using the DNeasy Tissue Kit (Qiagen, UK). Amplification was performed using Thermo-Start Taq DNA Polymerase (ABgene). dNTP mix was supplied by Novagen Ltd (10mM each dNTP). Primer sequences and reaction conditions were from previous publications. PCR products were loaded onto a 1.5% agarose gel and visualized using ethidium bromide.

RNA extraction was performed on frozen tissues using RNeasy kit (Qiagen, UK). RNA quality and quantity was measured using a Nanodrop spectrophotometer (Nanodrop Technologies, USA). RT-PCR was performed using equivalent amounts of total RNA and gene specific primers towards GAPDH.

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## Results and Discussion

Microscopic assessment confirmed equivocal morphological preservation and staining to that of formaldehyde-based fixatives as demonstrated in figures 1 and 4. Importantly, no tissue shrinkage was seen and nuclear detail was more crisp. Traditional histochemical stains applied to the liver samples were generally no different, although the use of the periodic acid-Schiff (PAS) reaction for demonstration of carbohydrates gave a more intense stain when FineFIX was used.

Immunohistochemical staining was also similar. Some antibodies such as CD117 which with formaldehyde-based fixatives gives a diffuse, cytoplasmic staining showed a stronger more distinct pattern (figure 4). Some of the T and B-cell markers were not as intense, but once optimised were equivocal. It therefore suggests that optimisation would be required as with any change of fixative or processing regime. To date over 50 routine diagnostic antibodies have been evaluated.

More importantly, yield and purity of DNA and RNA was radically enhanced by using FineFix. PCR (polymerase chain reaction) was possible for sequences not amplifiable from formalin-fixed tissue and RT-PCR gave comparable results to that of snap frozen tissues (figures 2 and 3). In diagnosis of T-cell gene rearrangements, KIT mutations in GIST tumours, HER2 gene amplification in breast cancer to name but a few, the use of FineFIX may prove to be invaluable. Increasingly there is a demand from Oncologists to know of any changes occurring at a gene specific level so that appropriate drug treatment can be administered to patients.

Our study, although ongoing, demonstrates that FineFix is the way forward for fixation of tissues, within the modern pathology department.

Figure 4: GIST

