Letters to the Editor

Examination and recording of large format histopathological specimens

A substantial volume of the workload of our laboratory concerns the production of large format histological sections from osteo-articular specimens of large size and complex shapes. The sheer size of the resultant glass slides makes them difficult to examine microscopically. We have overcome this difficulty by using either a Durst M605 colour enlarger or a De Vere 504 black and white enlarger. Placing the glass slide in the negative carrier of either of these allows an image (in full colour) to be projected onto the enlarger base board. The De Vere 504 enlarger can accept a maximum glass slide size of 13.5 × 11.0 cm, and the size of the projected area can be reduced by several standard masks available with the instrument. Large fields of view are obtained, and the magnification range is × 0.5–× 10. Small areas of the image can be examined in greater detail by a Paterson focusing telescope producing results superior to those obtained with a dissecting microscope.

To obtain a photographic record we discovered two useful strategies. Firstly, the glass slides were thoroughly cleaned and replaced in the negative carriers of the enlargers to produce positive images recordable as colour prints. After experimentation with several commercially available products we found the best results were obtained with Agfachrome-Speed 312 photographic material. This permitted better colour correction (with filters on the Durst M605 and Kodak colour compensating filters on the De Vere 504), and correct exposure times were more easily determined using test strips. The results were colour prints available within eight minutes by following the standard processing instructions supplied by the manufacturer.

The second strategy was to use the Agfachrome-Speed material to obtain colour photomicrographs of conventionally sized sections. The material was placed in a large format camera back on a Vickers M17 research microscope. Colour balance was achieved with Kodak colour compensating filters, the correct exposure quickly and easily obtained for each objective, and the print processed to become available within eight minutes.

These techniques avoid the necessity of purchasing specific equipment to make a 35 mm colour film and enlarging this to a

print. The advantages are that graininess in the print is reduced and there is superior resolution. We calculated that the cost of producing a print by this technique is virtually equal to that of the conventional technique of using 35 mm colour film, but there is a very large and advantageous time saving.

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Quality control of oestrogen receptor assays using frozen breast tumours

The value of oestrogen receptor status for predicting response to hormone treatment in women with breast cancer has been confirmed.^{1,2} Moreover, oestrogen receptor status is a prognostic factor and has recently been widely used in comparison with other prognostic indicators, such as epidermal growth factor receptor³ and serum concentration of carcinoembryonic antigen.⁴ The demand for biochemical assays of the oestrogen receptor status in breast cancer is now no longer restricted to research or clinical trials; in many centres these assays are now considered to be part of the clinical information necessary for the management of patients with such malignancy.

In the Grampian area the incidence of all types of breast cancer is about 150/600 000 per year. In past years receptor assays on breast tumours from this area have been carried out in the university department of clinical surgery, Edinburgh, after the specimens have been transported in dry ice. This was not ideal as local assays would provide receptor status more rapidly with less risk of loss of the labile activity during transport. A decision was therefore made to set up an oestrogen receptor assay service for Grampian, starting by showing that the local results achieved comparable accuracy and reliability with those achieved in the established centre. Although lyophilised samples of tissues have been widely used for inter-laboratory quality control,^{5,6} these materials do not generally test the tissue disruption step, and we therefore compared results obtained on 59 primary breast cancers collected in Aberdeen and tested in both centres.

The tissues were transported fresh from the theatre directly to the department of pathology, University of Aberdeen, where, af-

ter blocks had been taken for diagnosis, the remainder was frozen in liquid nitrogen. Portions of these tumours were then assayed in Aberdeen and sent on dry ice to Edinburgh. The method used was based on those of Korenman⁷ and Feherty *et al*⁸ with slight modifications.⁹ In brief, tissue (> 200 mg) was finely divided and homogenised in Tris buffer (10 mM Tris, 1 mM edetic acid, 0.25M sucrose, pH 8.0), containing mono-thioglycerol (1% v/v) and glycerol (10% v/v). After centrifugation at 2040 × g for 20 minutes portions (100 µl) of the low speed supernatant were incubated overnight with a fixed concentration of [³H] oestradiol—17β (0.031 nMolar) against different concentrations of non-radioactive oestradiol—17β (varying from 0.000 to 61/200 nMolar). Free and bound hormone fractions were separated by the addition of dextran coated charcoal suspension (0.5 ml 0.15% w/v Norit A charcoal and 0.0015% w/v dextran T70) and centrifugation at 1460 × g, and the bound fractions were counted in a scintillation counter. The data obtained were analysed in each centre according to the method of Scatchard.¹⁰ The total soluble protein content of each supernatant was determined, and receptor concentrations were expressed as fmol receptor sites/mg soluble protein. Values < 5 fmol/mg protein were regarded as negative in each centre.

Of the tissues examined, 15 were receptor negative in each centre, 40 were positive, and only four samples (all < 30 fmol/mg protein) showed discrepancy between the two centres. Two of these discrepant results, positive in Edinburgh (30 and 15 fmol/mg protein) but negative in Aberdeen, may have been due partly to difficulties with the computer analysis of low values on a fixed scale plot on the Aberdeen computer: subsequent manual plots yielded values of 7 fmol/mg and 12 fmol/mg protein, respectively. The third discrepancy related to a breast tumour, which had been left in the operating theatre overnight, while the fourth result was associated with a poor Klodolton in Edinburgh and, in retrospect, may have represented non-specific binding. The range of values found for the dissociation constant of binding was 0.013 to 3.547 × 10⁻¹⁰ molar in Aberdeen and 0.021 to 2.114 × 10⁻¹⁰ molar in Edinburgh. The figure shows the relation between the concentration of receptor found in each centre. There was an excellent correlation between quantitation in the two centres, the correlation coefficient being +0.97; the gradient of the line calculated by regression analysis was 0.98.

Our results show that a receptor assay, using solid tumours, has been satisfactorily