Letters to the Editor

Multiblock: an aid in diagnostic immunohistochemistry

Immunohistochemical analysis of tissue sections from formalin fixed, paraffin wax embedded material is now routinely used as a diagnostic tool in many pathology laboratories. The fixation and paraffin wax embedding routines can differ between laboratories. Such differences might influence the preservation of some antigens, and each laboratory must therefore carry out extensive and tedious testing of antibodies and labelling techniques. Furthermore, without well executed controls the immunostaining results cannot be safely interpreted.

Conventional histological techniques allow sections from only one tumour per slide to be stained on each occasion. Thus there is a need for a simple histological technique that increases the number of tumours and tissues on each slide. We describe a method that meets these requirements.

Using this method representative areas of tumours or tissues are delineated on routinely stained sections retrieved from the surgical pathology files. Corresponding areas in the paraffin wax blocks are then punched with a skin biopsy instrument (4 mm in diameter), modified with a mandrin

(figure). Multiblocks containing up to 30 punch specimens from different tumours or tissues, are made by placing the punched specimens in a warm cast containing a small amount of melted paraffin wax. The position of each punch specimen in the multiblock is recorded.

When evaluating a new antibody, five to 20 pre-anticipated positive and negative punch specimens are selected for a multiblock, primarily to determine the most suitable dilution.

To test the specificity of the antibody, multiblocks are created comprising mainly tumours of differential diagnostic importance. Two to three hundred tumours can be immunostained at once. A new multiblock is designed for each antibody. Tumours showing various degrees of positivity and negative reactive tissues are chosen for this purpose. Sections of the specimen to be immunostained are then mounted on the same multiblock section slide (figure) containing the necessary controls. Five to six punch specimens are often sufficient in a "control multiblock" and thus reduce the amount of antibody necessary for coverage.

We have initially tested this method using a few polyclonal antibodies (for example S-100, Dakopatts AG) and an avidin-biotin complex method (Zymed). Up to 240 different tumours were successfully immunostained at once.

A somewhat similar method, mainly desig-

ned for testing hybridoma supernatants, has recently been described by Battifori.¹ His multitumour tissue block method requires deparaffinisation and rehydration of the tissue, which is cut with a razor blade into 1 mm slender slices. Up to 100 of these tiny rods are then re-embedded into a new paraffin wax block. By contrast, our method requires no rehydration; the 4 mm punches ensure representative areas of each tumour; the punches are practical to handle; and individual tumours can easily be identified.

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Potential transport medium for *Campylobacter pylori*

Our experience with *Campylobacter pylori*, a new enteric pathogen associated with active chronic gastritis,¹ is that recovery after freeze-drying by standard procedures is extremely poor.² It has consequently been problematic to dispatch reference strains of *C pylori* on a world wide basis. Alternatives such as blood agar culture or suspension on dry ice also tend to give erratic recovery rates, are inconvenient, and expensive to despatch. To resolve this problem we investigated the possibility of using a semi-solid agar as the basis of a transport medium for *C pylori*. We report here the results on the survival of two reference strains (NCTC 11637 and NCTC 11639) in three different media.

The strains were cultured on 5% v/v horse blood agar for four days at 37°C under microaerobic conditions, then suspended in 5 ml sterile distilled water. The following media were tested: Stuart's medium³; semi-solid motility test medium (SMTM)⁴; containing Oxoid Lab-Lemco powder (0.3% w/v); and brain heart infusion medium (BHIM), comprising Oxoid brain heart infusion broth, 0.5% w/v agar, and 7% v/v horse blood. BHIM is similar to the medium that Goodwin *et al*⁶ used to maintain *C*

Figure Multiblock technique. Left: Routinely stained slide with delineated representative tumour area and the corresponding punched paraffin wax block. Centre: Punch specimen and the skin punch biopsy instrument modified with a mandrin. Right: Multiblock and a slide containing a specimen section (S) plus a section from a multiblock designed as control for an antibody (CLA). The number of punch specimens can be reduced in this multiblock to save antibody.

Figure Comparison of survival patterns

C. pylori but has a higher agarose concentration, contains whole horse blood instead of horse serum, and excludes yeast extract. Six ml volumes of each transport medium in glass phials were inoculated with cotton wool swabs previously moistened with the bacterial suspension (about 10^6 cfu). The cultures were stored under three different temperature conditions: 4°C, room temperature (18–22°C), and one day at 37°C (to initiate bacterial growth), followed by room temperature. After the required period swabs were used to subculture from each medium on to horse blood agar plates. A semiquantitative estimate of viable bacteria was made from the correlation between Miles-Misra and spread-plate counts.

We found that *C. pylori* was not recoverable from Stuart's medium, while different levels of survival were observed in the other two media (figure). Both strains gave optimal survivals in BHIM, although strains survived in SMTM. There was no recovery after 14 days' storage in either medium. Although NCTC 11639 grew better than NCTC 11637, both strains were recovered from BHIM after storage for 10 days; the level of viable count depended on the storage temperature. There seemed to be no particular advantage in incubating at 37°C before storage.

We suggest from these results that BHIM at room temperature or 4°C may provide a convenient and effective medium for transporting *C. pylori* under most circumstances. It is essential that subcultures are made

within eight to 10 days after the initial inoculation and that an extended incubation period (four to six days) is allowed for recovery of *C. pylori* following cell stress incurred during storage. We emphasise that the medium has been tested using strains which were well adapted to laboratory cultivation and needs to be evaluated with recent clinical isolates as well as reference strains under the more variable conditions likely to be encountered in transportation.

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Nucleolar organiser regions in lymphocytes of patients with chronic lymphocytic leukaemia

Nucleolar organiser region associated protein sites (AgNORs) are found in the nucleoli of cells which possess genes for ribosomal RNA (rRNA). They can be identified by a simple one step silver colloid method which was first applied to paraffin wax sections to distinguish between low and high grade non-Hodgkin's lymphoma.¹ This has further been used to investigate melanocytic lesions of the skin,² breast lumps, and fibrous proliferations in childhood, among other tumour types. AgNORs probably reflect the activity of the cell and may be related to the degree of malignancy. There is a high correlation in non-Hodgkin's lymphoma between the numbers of AgNORs in each nucleus and labelling with the mono-

clonal antibody Ki67 (a proliferation marker) and the number of S phase cells, as measured by DNA flow cytometry. Chronic lymphocytic leukaemia (CLL) is characterised by the presence of increased numbers of small lymphocytes (immunologically MRBC + TU1 + SIg + (low density) FMC7–). A proportion of cases have morphological and immunological features intermediate between CLL and B cell prolymphocytic leukaemia (CLL-Pro).^{3,4} These patients may present with these features or develop them during the course of the disease and they are associated with a refractory response to treatment and a poor outlook.

We examined the peripheral blood of 30 patients with CLL. One of the authors assessed "blind" the percentage of "prolymphocytoid" cells in each of the smears and the number of AgNORs/100 lymphocytes was calculated as described previously.⁵ There was a wide range of AgNOR counts but no correlation was shown to exist between AgNOR numbers and prolymphocytic progression of CLL. Regression analysis gave a correlation coefficient (r) value of 0.23.

Despite its value in the investigation of other tumours we conclude that AgNOR determination is unhelpful in the distinction between CLL and CLL-Pro. It remains to be seen whether a better correlation exists between AgNOR numbers and cell surface immunological expression that does not necessarily correlate with the morphological appearance.⁵

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