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 designed for testing hybridoma supernatants, has recently been described by Battifora. 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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Reference


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 medium;3 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 al5 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.

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