CORRESPONDENCE

The precious cell block

We describe a method for preparing a cell block from a very sparsely cellular cytology fluid sample. The aim is to ensure that cells are not lost during processing. We developed this method to perform immunohistochemistry on circulating tumour cells (CTC) extracted from the blood of patients with metastatic carcinoma, in which there are typically less than 20 cells in a 10 mL blood sample. The method could also be applicable to other sparsely cellular samples such as cerebrospinal fluid (CSF) samples, or eye vitreous fluid samples.

First, the cells are concentrated into a 90 µL volume of fluid. In the case of CTCs this is obtained from a phosphate buffered saline backwash of a Parsortix CTC blood filter cassette (figure 1A–C; arrows mark CTCs). This filter cassette separates cells based on their size and deformability. Circulating carcinoma cells, and cells from other non-haematological neoplasms, tend to be larger and more rigid than other blood cells. The concentrate could also be from centrifugation of a CSF sample, vitreous humour or similar, with removal of most of the supernatant and resuspension.

Next, a 40 µL droplet of plasma or serum is placed at the centre of a clean glass microscope slide (figure 1D). In the case of CTCs, the plasma is derived from the patient's own blood sample, but fetal calf serum could be an alternative. A 12 mm Cytofoam Disc is placed on top of the droplet on the slide and the 90 µL sample containing the cells is added to the centre of the top face of the disc (figure 1E). A further 40 µL of plasma or serum is then added (figure 1F). The surface of the disc is then prodded with a micropipette tip 30 times to encourage mixing of the fluids. A 500 mL plastic histology specimen container, or similar, is then prepared with a folded paper towel across its floor that is soaked with 5 mL of neutral buffered formalin. The glass slide is then placed flat on top of this with the Cytofoam Disc uppermost (figure 1G). The container's lid is closed and left at room temperature for 24 hours for the sample to fix in the formalin vapour. The slide is then removed from the container and Cytofoam Disc prised from the surface of the glass slide with the edge of a scalpel. The disc is then wrapped in tissue paper and paraffin processed as for a biopsy specimen. The discs are 2 mm thick in the unprocessed state, but less thick when processed, and sometimes slightly concave or convex. We are reliably able to cut 50–80 paraffin full face sections, 3 µm thick, from each cell block and place five sections on each standard histology slide. In this way, we are able to perform immunostains for at least 10 antibodies, to attempt to characterise the cells of interest. Having five sections on each slide (figure 1H) increases the number of cells that are seen for each immunostain. It might be possible to use gelatin foam (eg, Gelfoam) as a substitute for CytoFoam.

This technique allows routine H&E paraffin sections and immunohistochemistry to be used to characterise very sparsely cellular samples, and so makes it possible to analyse CTCs using the routine methods employed in most diagnostic histopathology laboratories. In the blood of some patients with metastatic carcinoma, CTCs can be recognised on immunohistochemistry (figure 1I), and some of these patients also have prominent circulating megakaryocytes (figure 1J).

It should be emphasised that the application of this method to the recognition and characterisation of CTCs in blood is not currently at a stage where it could be used in routine clinical practice. We are conducting a study of 50 patients with known, or suspected, metastatic carcinoma to assess the potential diagnostic utility of this method.

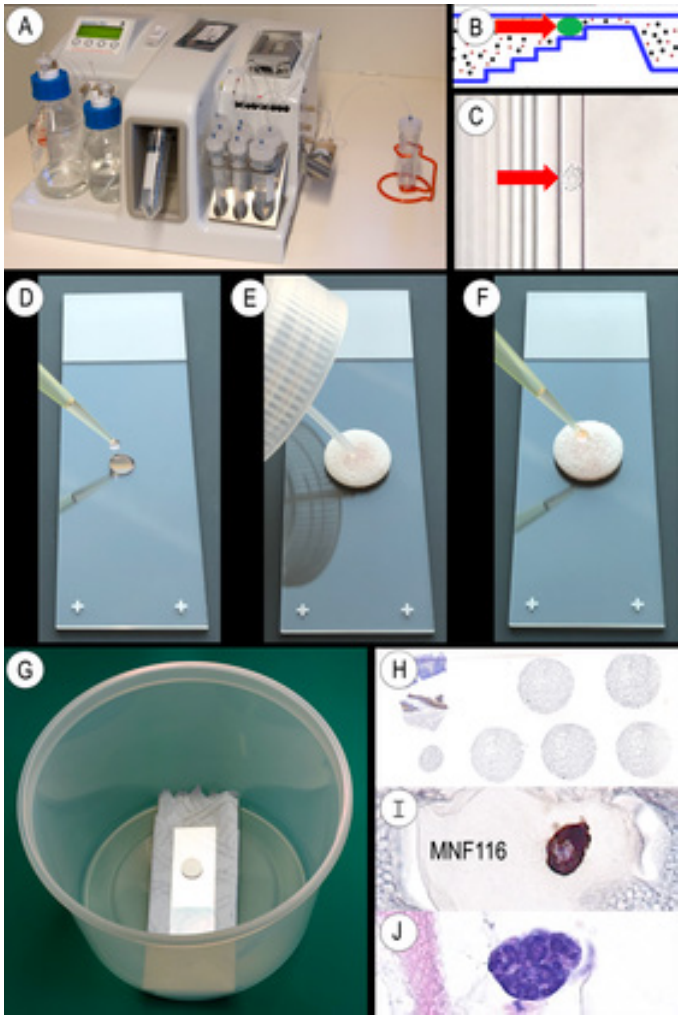

Figure 1 A montage demonstrating the Parsortix blood filter technology (A-C), the cell block preparation method (D-G) and the resultant histological and immunohistochemical sections (H-J).

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