
Affinity Blotting: Polyvinylidfluoride (PVDF) Is Superior to Nitrocellulose, S. Callaghan and R. Mc7lan (Pathol. Dept., Peter MacCallum Cancer Inst., 481 Little Lonsdale St., Melbourne 3000, Australia)

Affinity blotting is a simple and rapid technique for characterizing monoclonal immunoglobulins present in low concentrations in body fluids (1). After electrophoretic separation, proteins are selectively bound to an antibody-coated nitrocellulose (NC) membrane and subsequently detected with specific enzyme-labeled antibodies.

The frequent incidence of artefacts due to uneven coating of the NC membrane prompted us to evaluate polyvinylidfluoride (PVDF; Millipore Pty. Ltd., Sydney, Australia) as an alternative medium. The use of PVDF overcame troubling artefacts that hindered interpretation of results, allowed us to shorten some time-consuming steps, and improved the sensitivity of protein detection. PVDF membranes are also more robust than NC, thus allowing easier handling throughout the procedure, particularly during the coating step.

We modified the coating, transfer, and first wash steps of the method recently described (1), reducing the assay time by 40 min. After prewetting the PVDF membrane in 100% methanol for 1-2 s, we rinsed it briefly in distilled water followed by 10 mmol/L phosphate-buffered saline (PBS), pH 7.5, and blotted it on filter paper. After layering the membrane onto the antibody solution, we immediately dried it under hot air. The procedure took <5 min. The affinity membrane may be stored until required (at least three weeks), but must be re-wet as above before use. Proteins were separated by either of the techniques previously reported, and the membrane was blocked as described (1). The capillary transfer of proteins to the affinity filter was complete in only 5 min. After removing the membrane from the gel with the aid of a little PBS applied to its surface, we placed it, without washing, into appropriate antibody solutions. The detection procedure was unchanged.

A polyclonal IgG standard (Behringwerke AG; OTRD 03, Batch 046419B, 198 units/mL) was used to assess the limits of sensitivity of the modified PVDF method. The detection limit was increased threefold over that of the NC method under identical antibody and staining conditions. The lower limits for detection of polyclonal IgG were as follows: for NC, 10 milli-units, equivalent to 14 ng of IgG; for PVDF, 3 milli-units, equivalent to 4.2 ng of IgG. It was not possible to detect the low quantities of IgG previously seen for a monoclonal IgG standard (Behringwerke AG; OTRD 03, Batch 046416C, 204 units/mL) (1), because of the comparative lack of resolution of polyclonal immunoglobulins by isoelectric focusing. There is no doubt, however, that the use of PVDF membranes further enhances an already exquisitely sensitive technique.

Reference

Discordant CA 125 Measurements in Normal Healthy Women, Takuma Hashimoto, Noriko Obha, and Fujitsugu Matsubara (Dept. of Lab. Med., Kanazawa Univ. Med. School, 13-1, Takara-machi, Kanazawa, 920, Japan)

Several researchers have reported interference from circulating antibodies in human serum samples in immunoassay procedures. Two-site immunoradiometric assays (IRMAs) are particularly prone to interference by antibodies, because any agent capable of cross-linking the labeled antibody with the antibody on the solid phase can generate a false-positive signal in the absence of antigen (1-6).

In 1988 at the start of our routine work, we carried out a parallel study to compare the efficiency and reliability of the only two available enzyme immunoassay (EIA) and IRMA kits for CA 125. We used the Centocor IRMA (Centocor Co., Malvern, PA) and the Fujirebio EIA, Imnclone CA 125 method (Fujirebio Co., Tokyo, Japan). Using a monoclonal antibody (Ma1 OC125, purchased from Centocor Co.) for CA 125, we found a marked discrepancy in the results for CA 125 in the serum of seven of 397 subjects tested (including 178 normal healthy subjects). The CA 125 EIA kits were used according to the manufacturer's instructions. First, 50 μL of serum or standard CA 125 solution (30-480 arb. units/mL) was added to the polystyrene beads (outer diameter, 6.4 mm, coated with mouse anti-CA 125 antibody) contained in a tray, followed by 150 μL of 0.1 mol/L Tris buffer (pH 8.2, containing bovine serum albumin, 10 g/L) and incubated for 2 h at room temperature. After incubation, the reaction mixture was aspirated and the beads were washed three times with saline. Then, 200 μL (0.2-0.8 mg/L) of enzyme-labeled monoclonal antibodies (horseradish peroxidase-labeled anti-CA 125 antibody) was added and incubated for 2 h at room temperature. Again, the reaction mixture was aspirated, and the beads were washed four times with saline, transferred to test tubes, and mixed with 400 μL of developer (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) in 0.1 mol/L Tris buffer (pH 4.4) containing 51 μL of H2O2 per liter) per tube. After incubation for 18 h at 4 °C, 2.0 mL of reaction stopper (5.4 g/L oxalic acid solution) was added, and the absorbance at 420 nm was measured photometrically.

Overall, CA 125 estimated by the Fujirebio EIA method (y) correlated closely (r = 0.983) with that estimated by the Centocor IRMA method (x): y = 0.54x + 3.5, n = 390, range 0-480 arb. units/mL. Three healthy women, however, had values of 556, 250, and 124 arb. units/mL by the EIA method, but had normal values, i.e., 31, 25, and 30 arb. units/mL, by the IRMA method. These females were gynecologically normal, and ultrasound examinations showed no evidence of pelvic tumor. Their menstrual cycles were regular. No clinical evidence of malignancy was present across the years after the initial discovery of their above-normal CA 125. We determined their CA 125 concentrations several times during their menstrual cycles over...