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Affinity Blotting: Polyvinyldifluoride (PVDF) Is Superior to Nitrocellulose, *S. Callaghan and R. McLachlan* (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 prompted us to evaluate polyvinyldifluoride (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 IgD 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 IgD were as follows: for NC, 10 milli-units, equivalent to 14 ng of IgD; for PVDF, 3 milli-units, equivalent to 4.2 ng of IgD. It was not possible to detect the low quantities of IgD previously seen for a monoclonal IgD 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.

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Discordant CA 125 Measurements in Normal Healthy Women, *Takuma Hashimoto, Noriko Ohba, 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, Imnoclone CA 125 method (Fujirebio Co., Tokyo, Japan). Using a monoclonal antibody (MAb 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-ethylbenzoline-6-sulfonic acid) in 0.1 mol/L Tris buffer (pH 4.4) containing 51 μL of H_2O_2 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 eight months after the initial discovery of their above-normal CA 125. We determined their CA 125 concentrations several times during their menstrual cycles over

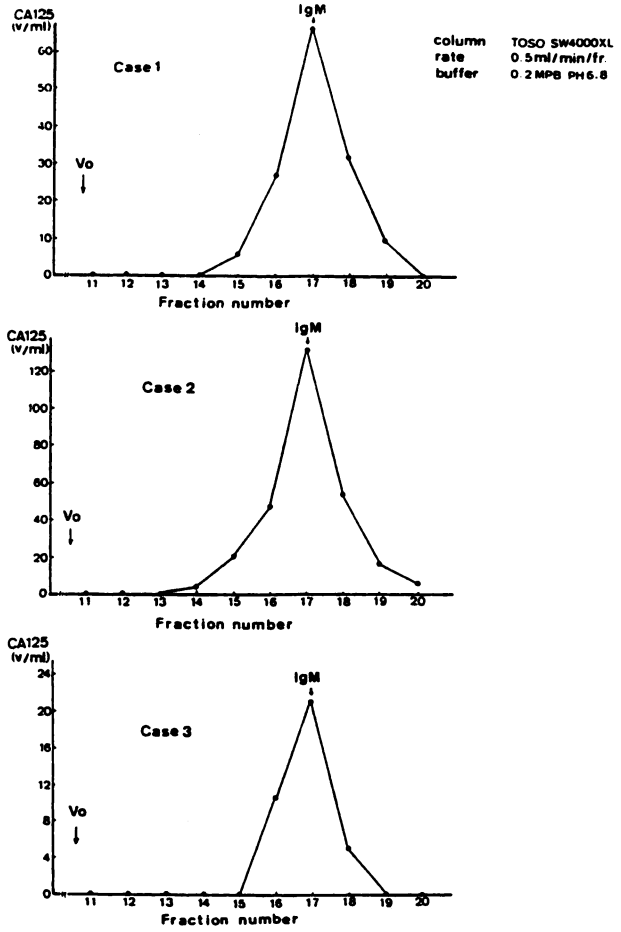

Fig. 1. HPLC elution profiles of assayable CA 125 activity in the three women with above-normal results

500 μL of an affinity-purified fraction from each subject's serum (containing 556, 250, or 124 arb. units of apparent CA 125 activity per milliliter) was applied to a 300 × 7.8 mm (i.d.) TSK G4000SW × L column (Tosoh Co., Tokyo, Japan), and 500-μL fractions (0.5 min per fraction) were collected for assay in 0.2 mol/L phosphate-buffered saline, pH 6.8. The elution time for the apparent CA 125 activity is shown relative to the elution times of peak (fraction 17: M_r 8 × 10^5 to ~1 × 10^6). CA 125 antigen was eluted with the void volume (V_o; M_r 7 × 10^6)

eight months, but none exhibited pronounced variations in CA 125 concentrations.

In trying to find the reason for this anomaly, we first checked our tests by using antibody-coated beads from the IRMA kit in the EIA. However, the values stayed high (522–117 arb. units/mL). These results indicated that the antibody-coated beads in the EIA were not responsible for this phenomenon.

Then we repeated our tests by using ^{125}I-labeled OC125 (purchased from Centocor Co.) instead of enzyme-labeled antibody; this yielded normal values (20 arb. units/mL) even with the Fujirebio antibody-coated beads. These results clearly indicated that the enzyme-labeled antibody is responsible for this phenomenon.

We speculated that the discrepant results for CA 125 observed in these women were ascribable to differences in the composition of and the methodology used in the two CA 125 assays. The Centocor two-site IRMA involves an Iodogen-labeled MAb tracer for OC125, which eliminates false-positive effects from circulating IgM (*7*). The Fujirebio EIA method involves only MAb OC125; lacking the Iodogen-labeled tracer, it is vulnerable to the IgM effect. In an attempt to clarify the discrepancies, we tried an HPLC study, which confirmed our speculation that the discordant CA 125 results were distorted by the IgM effect (Figure 1).

After this study, Fujirebio improved the EIA kit by Iodogenizing the antibody-coated beads, which eliminated the IgM effect. Overall, regression analysis of the results from 98 samples for CA 125, which now fell within the range of 0 to 335 arb. units/mL by both methods (the Fujirebio improved method and the original method), yielded a correlation coefficient of 0.989. The regression line was calculated by comparing the improved method (y) = 0.897 with the original method (x): $y = 0.897x - 5.412$ arb. units/mL. Precision (within-run and between-run) was <3.8% and 4.5%, respectively.

In conclusion, because many doctors and companies are involved in developing and producing EIA and IRMA kits for detecting CA 125 in their own laboratories (*8*), possible interference of IgM should be kept in mind when assessing results.

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Analysis of Convection Cooling Rates for Fluid Samples in Cryostorage

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Fluid samples are increasingly held in cryostorage for retrospective clinical and research purposes (*1–3*). The