Immuno-electrophoretic Quantitation of Alpha-Fetoprotein

M. Pietrogrande, L. Roffi, A. S. Bassi, and C. Vergani

We describe an immuno-electrophoretic method for alpha-fetoprotein quantitation. The sensitivity of the method, which is easy to perform in the routine laboratory, is under the best conditions 50 μg/liter, but 83 μg/liter can always be detected. With this test, sera from patients with various hepatic diseases and from pregnancies can be tested.

Additional Keyphrases: liver disease • cancer • teratoblastoma

Determination of serum alpha-fetoprotein (AFP) is useful in the diagnosis of hepatocellular carcinoma (1–3) and embryonal teratoblastoma (4, 5). The need for AFP quantitation has been stressed (6–9) in follow-up of patients who may be expected to have an increased incidence of primary hepatocellular cancer, for biochemical surveillance of patients with hepatomas subjected to chemotherapy or liver resection, and for evaluating severity of liver destruction and regeneration in acute hepatitis.

Modifications in the concentration of AFP have been described in maternal serum and amniotic fluid in the presence of abnormal pregnancies and fetal neural-tube defects (10–12).

Here we describe an immuno-electrophoretic method for AFP quantitation with which we can detect as little as 50 μg of the protein per liter under the best electrophoretic conditions; under ordinary conditions, 83 μg/liter can always be detected.

Materials and Methods

Pooled rabbit anti-AFP sera, commercially available (Behringwerke, Marburg-Lahn; batch No. 2864 G), were used. The specificity of the antisera was investigated by standard mono- and bidimensional agar gel immuno-electrophoresis. Pooled goat anti-rabbit gamma-globulin serum was used as “sandwich” antibody, to increase the sensitivity of the test.

The calibration curve was constructed by diluting standard AFP supplied by Behringwerke (25 mg/ml).

Complete identity was seen on immuno-electrophoresis and immunodiffusion between standard AFP and AFP from hepatoma and amniotic fluid collected at the 20th week of gestation.

Procedure. Pour 8 ml of agarose (8 g/liter) in diethylbarbiturate buffer (pH 8.6, 20 mmol/liter onto 11 × 21 cm glass slides, previously coated with agarose (3 g/liter), to give a slab of gel 1.5 mm thick. After the agarose has solidified, cut it with a razor blade, leaving a slab 3 cm wide on the glass plate. The lift-out space is filled with agarose (8 g/liter) in the buffer containing 1.5 ml of anti-AFP serum per liter. Punch holes with a 4-mm diameter puncher in the antibody-free gel, 3 mm from the antibody-containing gel. Fill the holes with 15 μl of sample, using a Hamilton microsyringe or double-constriction pipettes. Electrophoresis in the buffer at 15 V/cm of gel for 6 h in an electrophoretic chamber cooled with a coil containing cold water. Then wash the slides in phosphate-buffered saline for 24 h to remove unprecipitated proteins. To improve the sensitivity of the test uniformly spread 200 μl of goat anti-rabbit y-globulin on the gel with a glass rod. Leave the plate at room temperature for 10 min and then transfer into phosphate-buffered saline for 24 h. Stain the dried gel with Coomassie Brilliant Blue. Plot the standard calibration curve on linear paper in the range 50 to 400 μg/liter and on semi-logarithmic paper in the range 0.4–2.5 mg/liter in order to obtain straight lines.

Results and Discussion

The AFP-positive sample gives a rocket precipitation line in the antibody-containing gel. A clear precipitate is always visible up to 83 μg/liter, corresponding to a standard dilution of 1:3000. At a dilution of standard AFP of 1:5000 (50 μg/liter), a precipitate can be observed in 75% of the slides (see Figure 1).

By this method, differences of ±25 μg/liter in AFP concentration can be detected. An AFP-positive reference serum run on 25 different days showed a coefficient

Istituto di Clinica Medica III, Università di Milano, Via Pace 15, 20122, Milano, Italy.

Received Sept. 27, 1976; accepted Nov. 24, 1976.
of variation of 6.0%. The reproducibility of the test was determined by running a sample four times on the same slide; the resulting intra-assay coefficient of variation was ±4.6%.

We investigated 220 patients with liver disease. Diagnosis was made from histological or biochemical data. Sera to be quantitated for AFP are run initially undiluted. Samples containing >2.5 mg of AFP per liter must be diluted in buffer in order to bring it into a measurable range. Table 1 summarizes our results.

This method, which improves the sensitivity of Laurell's technique, is easy to perform in the routine laboratory. Concentrations of AFP ranging from 83 to 2500 µg/liter can be determined on the same plate in undiluted samples. The test is a simple, useful tool for studying and monitoring hepatic diseases and pregnancies with low concentrations of serum AFP.

References