Technical methods

Method for the detection and identification of alpha fetoprotein in serum

J. KOHN *From Queen Mary's Hospital, Roehampton, London*

The value of the demonstration of alpha$_1$ fetoprotein (α_1 FP) in the diagnosis of hepatoma is now well established[1] (O'Connor, Tatarinov, Abeleve, and Uriel, 1970; Purves, Bersohn, and Geddes, 1970). The technique most commonly used for this purpose is bidimensional immunodiffusion which is simple and practical, but may take up to 72 hours and is not very sensitive. The complement-fixation test and radial immunodiffusion have also been used. The former is more sensitive but it is rather difficult to establish its specificity whereas the latter requires relatively large quantities of antiserum. Radio-immunoassay involves isotope labelling.

This paper describes a technique for the detection and identification of α_1 FP in serum by means of counter-current (cross-over) electrophoresis in agar gel (Bussard, 1959; Culliford, 1964, Kohn, 1967). The specificity of the precipitation lines is established by a reaction of identity with a positive control incorporated in the test system. Electrophoresis is performed on agar gel with the antigen sample on the cathode and the antibody on the anode. Under these conditions the reactants are propelled towards each other. The speed and sensitivity of the test greatly increases.

Agar plates are prepared as for conventional immuno-electrophoresis. Lantern slides (7 × 6 cm or 8 × 8 cm were used) are covered with a 1 mm layer of 1·25 % agar in barbitone buffer *p*H 8·2 (sodium barbitone 47·6 g in 4·3 l distilled water add 69 ml N HCl). Oxoid I.D. agar is used throughout, but other brands are probably equally satisfactory. Agarose does not seem to offer any particular advantage. Sodium azide 1:1,000 is added as a preservative. Circular wells are cut in the agar layer along the electrophoretic axis approximately 5-6 mm apart (Fig. 1) midway between cathode and anode; the exact position is not critical. The antigen (patient's serum) wells are approximately 7 mm in diameter, holding about 50 μl. The antibody, ie, anti-α_1 FP wells, are smaller, approximately 4 mm in diameter, and hold about 15 μl. The size of the wells is not critical and may have to be varied according to requirements, eg, antibody titre of antisera. A micro technique using scaled down well sizes also gives satisfactory results and is only slightly less sensitive. A Culliford cutter was tried, but the pattern does not allow for controls. The wells are filled to the brim with a suitable micro pipette or thin capillary. It is advisable and indeed necessary to run tests on a series of dilutions of patient sera. This will prevent negative results due to excess antigen and will also provide semi-quantitative estimation of the antigen content. Specific and reliable anti α_1 FP antisera are now much easier to obtain from various sources and have recently been made commercially available.[2] The agar plate is placed on the bridge (4-5 cm gap) of an electrophoretic tank and connexion with the buffer solution is established by means of filter paper, lint, or agar. Barbitone buffer, *p*H 8·6, 0·05M, as for conventional electrophoresis, is used in the tank. A constant current of approximately 1·2 to 1·5 milliamps/cm width of agar plate, resulting in an initial potential of approximately 20V/cm (at the terminals), is recommended. Under these conditions precipitation lines become visible within one and a half to two and a half hours. The most important modification of the original technique consists in the introduction of controls. This is achieved by having two cathode (antigen) wells cut next to each other and the anode (antiserum) well positioned in such a manner that it is equidistant from both cathode wells (Figs. 1 and 2). (It is most important that the agar gel between the adjacent cathode wells is not damaged or lifted.) This procedure allows the precipitation line to be compared with that of a known positive control. Cord blood serum is an excellent control. It should be used at the greatest dilution which still produces a clearly visible precipitation line. After electrophoresis the agar plates can be photographed or, perhaps more conveniently, after visual inspection and recording of results, they can be washed overnight in 3% NaCl, dried and stained in the usual manner for immunoelectrophoresis. Faint precipitation lines are more prominent after staining. An enlarger or contact printing will provide excellent copies on photographic paper (Figs. 2 and 3). This procedure is much simpler and less costly than the various systems of dark ground illumination photography.

The advantages of the proposed method are (1) speed, as results can be obtained in one and a half to two hours; (2) greatly increased sensitivity, about five times compared with conventional bidimensional immunodiffusion, and α_1 fetoprotein can still be detected in foetal serum diluted 800 times; (3) the use of two adjacent

[1]These two papers contain up-to-date relevant references.

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[1]The Green Cross Corporation, 1/3 Gamaucho, Joto-Ku, Osaka, Japan, and Behring (Hoechst).

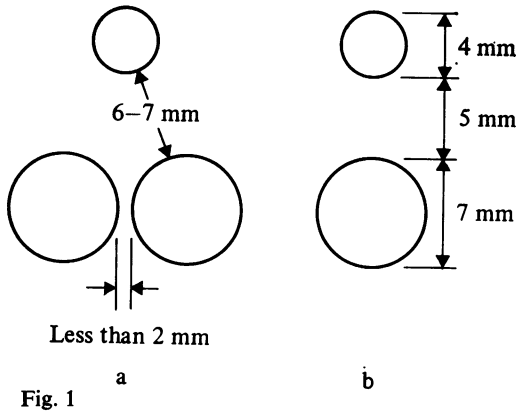

Fig. 1

Fig. 1 *Pattern of antigen and antibody wells.* (a) *Identification pattern with control in one of the wells. The same pattern can be used for different sera or serum dilutions.* (b) *Single pattern without control.*

Fig. 2 (a) *Positive control serum.* (b) (c) (d) (e) (f) (g) (h) *Increasing dilutions of serum from hepatoma case. The running together of the cathode wells is an artifact due to distortion during drying.*

Fig. 3 *Increasing dilutions of foetal serum run against anti* α_1 *FP (kindly supplied by Dr G. Schwick, Behring).*

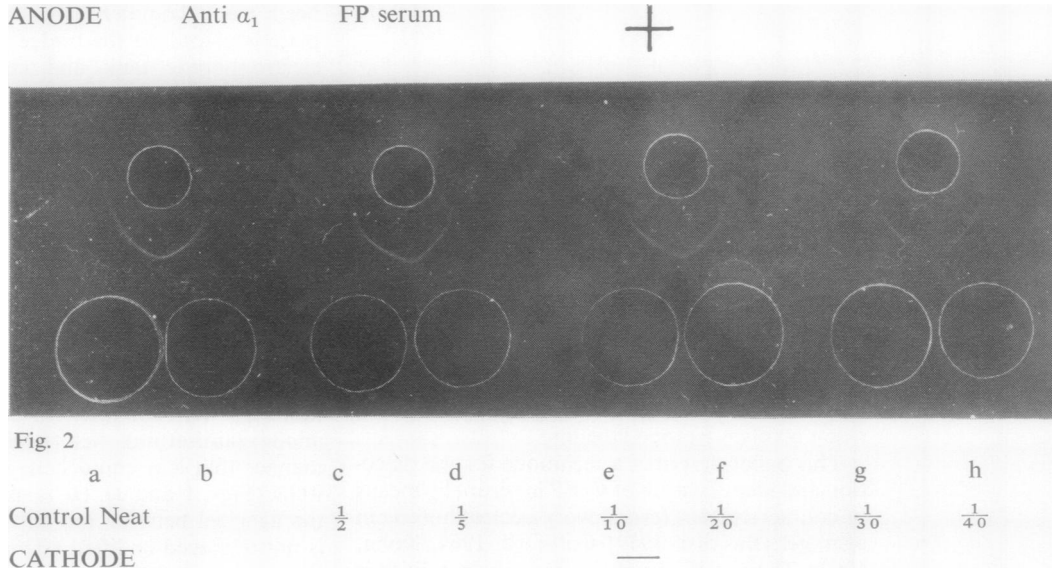

Fig. 2

a b c d e f g h

Control Neat $\frac{1}{2}$ $\frac{1}{5}$ $\frac{1}{10}$ $\frac{1}{20}$ $\frac{1}{30}$ $\frac{1}{40}$

CATHODE

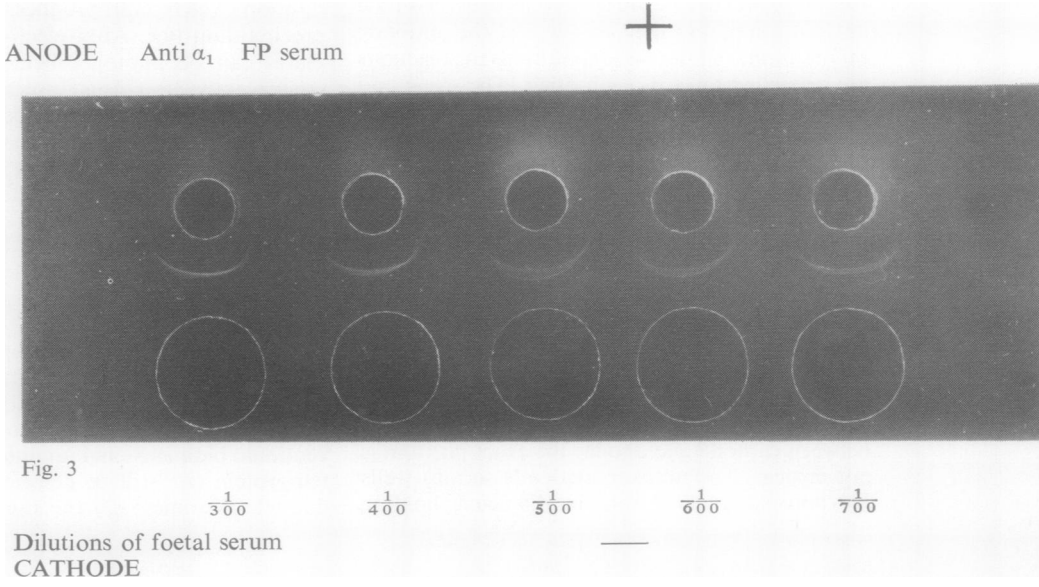

Fig. 3

$\frac{1}{300}$ $\frac{1}{400}$ $\frac{1}{500}$ $\frac{1}{600}$ $\frac{1}{700}$

Dilutions of foetal serum

CATHODE

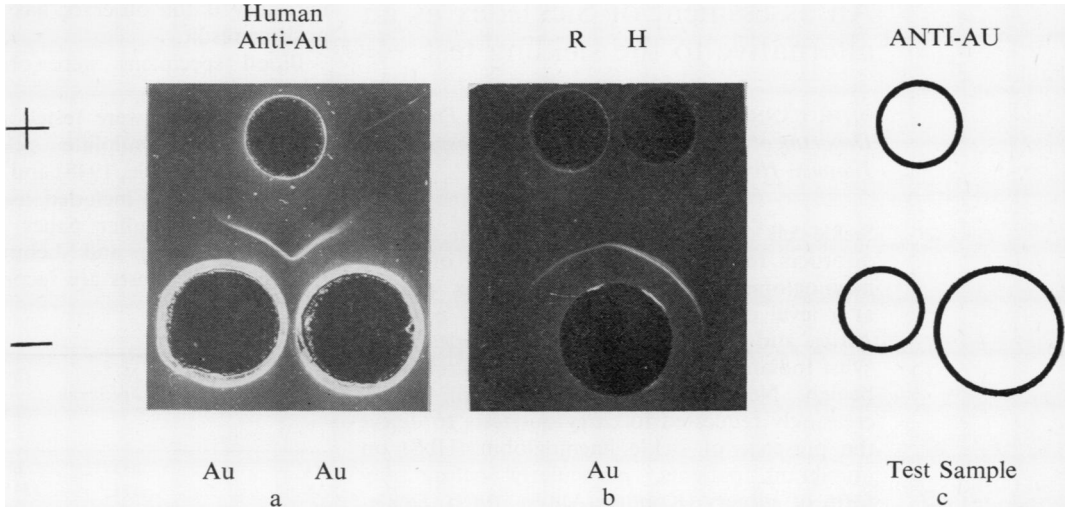

Fig. 4. *Demonstration of Australia antigen.*
(a) *Two Au-positive sera in cathode wells, human anti-Au in anode well. Note reaction of identity indicating specificity.*
(b) *Checking specificity of antisera. Au-positive serum in cathode well, R = rabbit antiserum (Behring), H = human antiserum in anode wells.*
(c) *This application pattern, allowing for widely different test sample volumes, helps to avoid false negative results due to antigen or antibody excess. This is very important in any unknown system.*

antigen wells, one containing a positive control, ensures specificity by establishing a reaction of identity of the precipitation lines; (4) a number of specimens can be tested simultaneously; and (5) economy of antisera.

It is suggested that the modification described above, *ie*, incorporation of a second antigen well, will also be very useful for other antigen-antibody systems where antigen has a mobility higher than gamma. This applies in particular to problems connected with identification of precipitation lines, eg, in forensic medicine, taxonomy, bacteriology, and in the investigations of cross reactions. The same modification should be most valuable when the counter-current electrophoresis technique is used for the detection of Australia antigen.

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