Detection of deficiencies in immunoelectrophoretic patterns

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When serum is examined by the usual immunoelectrophoretic technique an isolated deficiency may be overlooked due to the multiplicity of precipitin arcs and the confusion caused by overlapping arcs. To overcome these difficulties a simple method has been devised which takes advantage of the fact that antibody to a component which is not present in the test serum will diffuse beyond the region of the precipitin pattern and may be detected by reaction with a complete normal serum.

MATERIALS AND METHODS

Microscope slides (3 in. × 1 in.) are covered by 2 ml of 1.3% Ionagar² no. 2 in barbitone buffer, pH 8.2, $I=0.025$. The wells and troughs are cut as shown in Figure 1. The wells are 1.3 mm in diameter and are separated from each other by 2.0 mm. The troughs are 1.7 mm wide and each is separated from the nearest well by 2.5 mm.

One well is filled with the test serum and the other well with a known normal serum. Migration of the samples takes at a constant current of 5 mA per slide for 65 minutes. The troughs are then filled with antiserum to whole human serum and diffusion proceeds for three days.

RESULTS AND COMMENT

The manner in which deficiencies are demonstrated is shown in Fig. 1, in which the test serum (in the lower well) is umbilical cord serum. This sample is deficient in orosomucoid, haptoglobin, and IgA and the antibodies to these proteins have diffused across the agar to react with the relevant proteins in the normal serum (upper well). The appearance produced is that of a displaced arc which is made conspicuous by its lack of symmetry with the normal pattern. The normal serum thus performs a dual function: its primary function is to detect antibody which has not reacted with the test serum but it also forms a normal pattern for comparison. Using the method deficiencies of caeruloplasmin (in Wilson's disease) and IgA have been demonstrated in patients' sera.

The success of the method depends on the particular antiserum used. One of the two antisera which were used failed to demonstrate caeruloplasmin deficiency even though it produced a caeruloplasmin arc with normal serum.

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Loading starch gels using filter paper wicks

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Filter paper wicks are commonly used for loading protein samples into starch gels before electrophoresis. The usual procedure is to soak squares or strips of filter paper in the sample under examination and insert them into slits previously made along the line of application. Each wick is allowed to remain in the gel until electrophoresis is completed. We have examined this technique and

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FIG. 1. The troughs contain horse antiserum against whole human serum³. The upper origin well contains normal adult serum. Deficiencies of orosomucoid, haptoglobin, and IgA in the umbilical cord serum in the lower well are shown by the three displaced arcs. The displaced haptoglobin arc shows a reaction of identity with the haptoglobin arc in the normal serum pattern.

That the method will detect an incomplete deficiency was shown by adding 1 part of normal serum to 3 parts of caeruloplasmin-deficient serum: a displaced caeruloplasmin arc was still obtained.

A method based on the same principle has been used by Rabinovitz and Schen (1967) to detect $\beta_{1a/c}$ deficiency but in their method a deficiency is demonstrated by a single arc whereas normal serum produces no arc.

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REFERENCE

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