Present day practice

Pitfalls of Australia antigen detection and screening

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The recent spate of publications on cross electrophoresis (immunoelectrophoresis, electroimmunodiffusion, etc) techniques for the detection of Australia antigen prompts us to sound a note of warning. These techniques are rapid, simple, and sensitive but most of them suffer from the disadvantage of a fixed antigen to antibody ratio (equal size wells) and consequently do not take into account the possibility of false negatives due to either antigen or antibody in excess. This is particularly important when screening for Australia antigen as the titres of the antisera used are not standardized and vary within a wide range.

Attention has already been drawn to prozone phenomena occurring with immunodiffusion and complement fixation techniques (Schmidt and Lennette, 1970; Taylor and Zuckerman, 1970) and more recently with conventional cross-electrophoresis (Kohn, 1970).

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Prozone effects and consequently false negatives have indeed been encountered on routine screening for Australia antigen (Fig. 1). Strictly speaking the 'false negatives' are not all true 'prozone' effects, but may be due to the fact that precipitation lines in close proximity to the well edges are very difficult to detect. An obvious danger is that should antigen excess occur, high Australia antigen concentrations present in serum may be missed. It is most important to remember that a precipitation line obtained with a positive control does not necessarily make the test foolproof. With suboptimal conditions, some cases will be missed. A wrong antigen-antibody ratio or a brand of agar not suitable for a given system may still produce a false negative result. This error can be partly avoided by applying a series of test sample dilutions, but this procedure is both time consuming and limits the number of tests which can be performed simultaneously.

A simple, logical application pattern, recently proposed (Kohn, 1970) helps considerably to overcome the drawbacks of conventional cross electrophoresis. Essentially it is based on testing varying sample volumes in adjacent wells of different size and shape against the one antiserum. The optimal well sizes depend primarily on the type of antiserum used and are best established by experiment for a given system (Fig. 2). A further disadvantage of conventional cross electrophoresis is the difficulty in establishing the specificity of the precipitation lines obtained. The technique mentioned above with two closely adjacent antigen wells will permit also

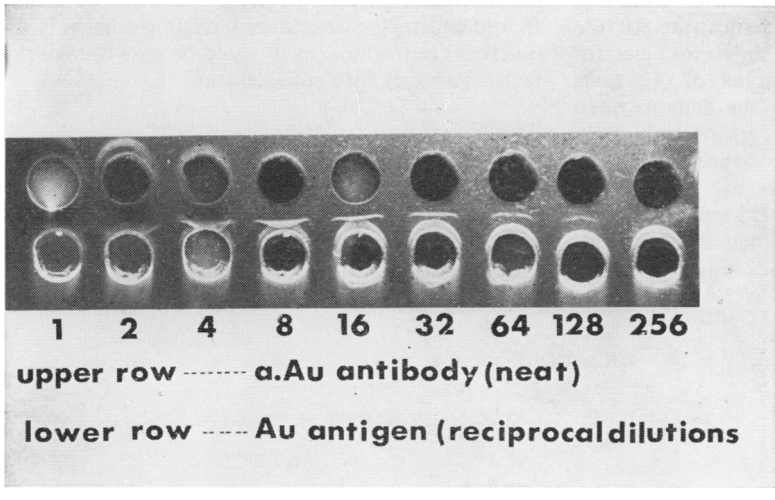

Fig. 1 *Prozone phenomenon using cross electrophoresis on agarose. Precipitate practically invisible in neat and 1:2 dilution of the test sample, clearly visible only between 1:4 to 1:64 dilution and again not beyond.*

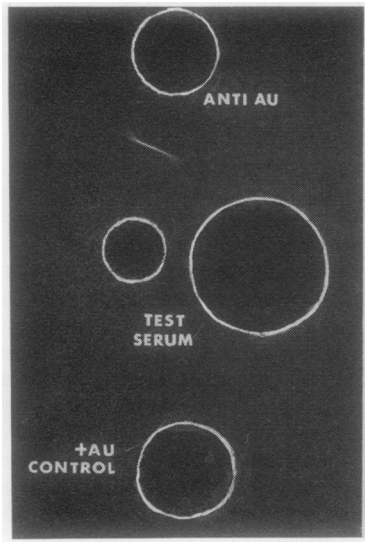

Fig. 2a

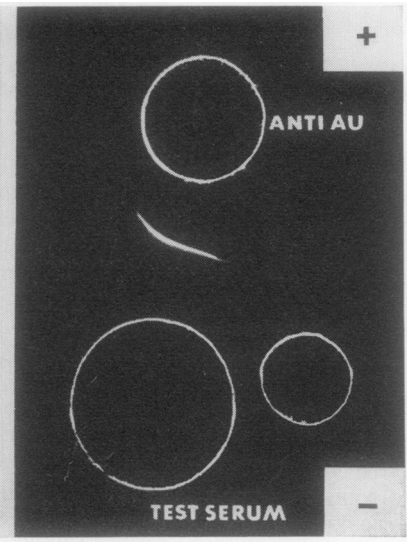

Fig. 2b

Fig. 2a *Strong antigen: precipitation line only visible against small test sample well. Practically invisible between large test sample well and antiserum well.*

Fig. 2b *Weak antigen: precipitation line only visible against large test sample well.*

Well diameters: *Antiserum well—3 mm, large sample well—4-5 mm, small 2 mm, sample well—Au positive—3 mm.*

identification of the precipitation lines by means of a reaction of identity with a positive control (Kohn, 1970). We are convinced that staining, admittedly more time consuming, definitely increases the sensitivity of the test and renders interpretation of the results much easier. This has been confirmed in other laboratories.

Cellulose acetate cross electrophoresis has some advantages but the sample volume is limited and application of a positive control close to the test sample would present considerable difficulties.

Another variable factor is the buffer and type of substrate used. Although agarose is becoming increasingly popular for immunodiffusion tests it is not necessarily the most suitable substrate for the detection of Australia antigen by cross electrophoresis. It was found on a number of occasions that Australia antigen could only be demonstrated on plain agar. Very satisfactory results have been obtained with an agar agarose mixture.

Errors may also arise if tests are carried out simultaneously for both antigen and antibody along the same electrophoretic axis. When this happens, the positive control on the cathode side can 'creep' past or through the test serum wells and react with the Au antiserum from the anode side moving in the opposite direction. Precipitation lines can thus appear between the test serum and the positive control, or even between the test serum and the antiserum, depending on the buffer used, conditions of run etc. Obviously a prolonged run is more likely to produce this type of false positive result.

Yet another word of caution. It is very likely that in the near future better quality, non-anti-complimentary antisera may be available. Consequently, tests like haemagglutination inhibition or complement fixation, which are much more suitable for mass screening, will become a practical procedure. Before embarking on a large screening scheme based on cross electrophoresis it would be wise to take this factor seriously into consideration.

References

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Schmidt, N. J., and Lennette, E. H. (1970). Complement fixation and immunodiffusion tests for assay of hepatitis-associated 'Australia' antigen and antibodies. *J. Immunol.*, **105** 604.

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