constant current of approximately 1·4 ma per cm width of agar plate is passed for a suitable time. Under these conditions α_1-fetoprotein gives visible precipitin lines within about one to two hours. The sensitivity of the test is considerably increased by subsequent staining. A positive result can be confirmed by means of a reaction of identity with a positive control (Kohn, 1970).

Figure 2 shows a reaction for α_1-fetoprotein obtained with cord blood serum. To illustrate the principle of the method two dilutions have also been tested, though routinely this would be unnecessary. Clearly, the undiluted specimen gave no reaction in the region where it was present at greatest excess, ie, at the right hand side in Fig. 2(A), whereas the 1-50 dilution gave a reaction in this region but not at the lowest concentration. (The dilutions refer to the serum before mixing with agar.) In this way, by observing the extent as well as strength of the precipitin line one is able to make a semi-quantitative assessment of the amount of antigen present.

Although we have used this modification only for detecting α_1-fetoprotein, it is presumably of general applicability.

The antiserum was obtained from Nordic Pharmaceuticals and Diagnostics (Tilburg, Holland). We wish to thank Mr D. H. Brown of the Physiology Department, University College, Cardiff, for constructing the gel punch, and the Department of Medical Illustration, Cardiff Royal Infirmary, for the photographs.

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Letters to the Editor

Rubella Screening Tests

We read with interest the article entitled 'A screen test for rubella haemagglutination-inhibition antibodies' (Thomas and Kempsell, 1972). In devising screening tests designed to save technician time it is always with regret that one accepts the necessity to repeat some of the tests, but we were surprised to read that 30% of the sera submitted to the screen test described had to be repeated in a full rubella HAI titration. The major difficulty appears to be lack of a clear-cut distinction between positive and negative sera at the dilution tested (1 in 32). A minor difficulty, but one which would be much more significant for anyone, like ourselves, who use pigeon RBCs, is the interference in the test of non-specific haemagglutinins. Our own screening test minimizes both difficulties, and is based on the following background observations.

Using the rubella haemagglutinin currently available from either Flow Laboratories Inc, Irvine, Ayrshire, or The Standards Laboratory, Central Public Health Laboratory, London, NW9 5HT, the removal of non-specific inhibitors of rubella-virus haemagglutination by kaolin according to the method of Halonen, Ryan, and Stewart (1967) is virtually instantaneous and is unaffected by the presence of pigeon RBC up to a final concentration of 3%. Moreover such a concentration of pigeon cells will remove the non-specific haemagglutinins of a 1 in 10 dilution of the vast majority of sera within 20 min, and this removal is marginally *more* effective in the presence of 12·5% kaolin. $MnCl_2$/heparin pretreatment is not used since in an HAI test involving overnight preincubation of virus/serum mixtures (see below) false positives are occasionally found with some antigens, and such pretreatment will always involve an extra step of further diluting the pretreated supernatants before placing in the microtitre wells.

Having pretreated sera with kaolin and pigeon RBCs the rubella HAI titres are determined in a microtitre system involving the mixtures of 4 units of haemagglutinin with serial doubling dilutions of serum using 0·4% bovalbumin-borate-saline pH 9·0 (Clarke and Casals, 1958) as both antigen and serum diluent. Virus/serum mixtures are then preincubated *overnight* prior to the addition of 0·25% pigeon RBCs in the RBC diluent of Stewart, Parkman, Hopps, Douglas, Hamilton, and Meyer (1967). Tests are then incubated at 4°C for one hr and read after 20 min standing at room temperature. The long preincubation period of virus/serum mixtures improves the distinction between positive and negative sera (see fig.).

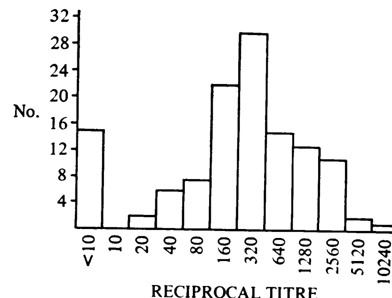

Fig. *Distribution of rubella HAI titres of 125 sera selected at random from those submitted from the antenatal clinic for screening.*

Based on the above, the following screening test procedure is employed. Serum, 0·1 ml, is mixed by hand shaking with 0·9 ml 12·5% acid-washed kaolin in borate-buffered saline pH 9·0 (Clarke and Casals, 1958) and 2 pasteur pipette drops (approx 0·065 ml) of 50% pigeon RBCs in RBC diluent. Mixtures are incubated at 4°C for 20 min with shaking after 10 min and then centrifuged at 3000 rpm for 5 min. Taking the supernatants as a 1 in 10 dilution the sera are screened at a dilution of 1 in 20 against 4HA units with a control lacking antigen for each serum. Virus/serum mixtures are then incubated overnight before adding the indicator RBC suspensions and developing the tests as described above.

Using this procedure we have found that less than 3% of screens require repeating, considerable time is saved since the duration of pretreatment of sera is reduced to 25 min and the partial haemolysis of pigeon RBCs in the presence of kaolin facilitates the placement of test sera in the colourless microtitre plates.

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Haemagglutination Test for Toxoplasmosis

We have read with special interest the paper by Thorburn and Williams (1972), since we were indebted to them for some technical advice when we were experimenting with pyruvic-aldehyde-treated red cells some time ago. Since then we have tested a large number of sera by this technique and have found our results similar to theirs. We too have been impressed with the possibility of using this technique for screening sera for toxoplasma antibodies, as a preliminary to carrying out titrations or other tests on those giving positive reactions.

Our work, which it is hoped to publish in due course, has largely been on the changes in titre in various serological tests in the course of toxoplasma infection. This has shown that in the first few months of a case of acquired toxoplasmosis it is normal for the HA test to have a much lower titre than the dye test, eg, the HA test may give a titre as low as 1/64 in the presence of a dye test titre of 1/8192. More important, we have had two examples of a negative haemagglutination test with a high titre dye test–one, a case of 'lymphocytosis' with a dye test titre of 1/2048, the other a child of 7 weeks with hepatosplenomegaly and a dye test titre of 1/4096. Dr Fleck also has reported two cases to us with the same type of serological response, one with our sensitized cells, the other with a commercial kit using a similar antigen. We have not encountered this discrepancy in eye cases, where it is usual to find the HA test yielding titres similar to or higher than the dye test.

We believe there is a good case for using this screening test in routine work but we hesitate to recommend it in suspected acquired cases (other than eye cases) of under six months' duration or in children under 1 year. Nevertheless, even in these cases a false negative result need only be expected on rare occasions.

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Book review

Methods of Biochemical Analysis Volume 19 Edited by David Glick. (Pp. vii + 632; illustrated. £9·85.) New York, London, Sydney, and Toronto: John Wiley and Sons Ltd. 1971.

The book starts with an admirable description of isoelectric focusing in LH gradients. This technique has come to the fore in the last two years in relation to the separation of proteins and of haemoglobins; it has an undoubted future in medicine and deserves more attention than it has received in pathological laboratories.

The second section deals with mass spectrometry in the determination of structure of certain natural products containing sugars, a very specialized field.

The third section deals with the determination of carbohydrate in biological materials by gas-liquid chromatography. To a worker in the field it is a valuable collection of references; it is not very critical and it is of little interest to anybody else.

The fourth section is concerned with activation analysis of the biological trace elements and is an uncritical compilation of the results of many authors: it has, of course, a very full bibliography.

The last section is a good description of the polarography of proteins. This technique of polarography has not been applied to clinical problems sufficiently for it to be possible to assess its value. It is a field which needs study and the present chapter would certainly be helpful to anybody seeking to study the subject.

It is impossible to resist the thought that books such as the present are unnecessary —what is worthwhile should appear as a review in a suitable journal and the rest should not be published.

ARTHUR JORDAN