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A SIMPLE METHOD FOR DETECTING ANTIBODIES TO RUBELLA

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Summary.—A simple microplate method of enzyme linked immunosorbent assay for Rubella antibody is described. This Micro-ELISA was compared with haemagglutination inhibition in a study of 188 human sera. The total discrepancy rate between the two tests was only 3·7%.

THERE is a need for a simple assay which can be carried out on a large scale for the detection of rubella antibodies, since the existing methods, complement fixation and haemagglutination inhibition, are not satisfactory in all respects, particularly because they involve so many manipulations of the sera.

In 1972 Engvall and Perlmann described an enzyme linked immunosorbent assay (ELISA) for the detection of antibodies and antigens. This communication deals with the application of a micro version of the assay to rubella.

MATERIALS AND METHODS

All the tests were carried out on Cooke microtitre disposable 96 well polystyrene plates (Type M29 AR, Dynatech Laboratories Ltd) sensitized with rubella antigen. The sensitization was carried out with soluble Rubella antigen (B. D. Merieux) diluted 1 : 200 in 0·05 mol/l carbonate buffer, pH 9·6. To each well of the plate 0·3 ml of the diluted antigen solution was added, then the plates were incubated at +4° overnight. The plates were then washed 3 times in saline containing 0·05% Tween 20 (saline-Tween) and after being shaken dry they were ready for use.

A human serum with a high titre in the rubella haemagglutination inhibition (HI) test was used as the reference positive serum and a human serum unreactive in the Rubella HI was used as the reference negative serum. These sera were diluted 1 : 10, 1 : 100 and 1 : 1000 in phosphate buffered saline, pH 7·2 containing 0·05% Tween 20.

One hundred and eighty-eight sera submitted for routine testing for rubella antibodies by HI at St George's Hospital, London, were retested by the Micro-ELISA at a dilution of 1 : 100. 0·3 ml amounts of the diluted serum samples were added to the wells of the sensitized plates and these were incubated for 2 h at room temperature. The plates were shaken dry then washed for 15 min in saline-Tween with 3 changes of washing fluid, after which they were again shaken dry. To each well 0·3 ml of a 1 : 700 dilution of alkaline phosphatase conjugated antihuman globulin (Engvall and Perlmann, 1972) was added. After 3 h incubation at room temperature, the plates were emptied and again washed for 15 min in saline–Tween. 0·3 ml of the substrate (p-nitrophenyl phosphate 1 mg/ml in 10% diethanolamine buffer, pH 9·8 with 0·5 mmol/l $MgCl_2$) was then added. After 30 min the reaction was stopped by the addition of 50 μl 2 mol/l NaOH to each well.

Positive reactions were recognized by the obvious yellow coloration in the wells. The results were assessed objectively using a spectrophotometer to measure the absorbance at 400 nm (E400) of the contents of each well. Readings above 0·2 were considered as positive.

RESULTS

The absorbance readings of the ELISA test on the reference positive and negative sera are given in the figure. At all dilutions, the positive serum gave much higher readings than the reference negative sample. In fact, the reference positive serum reading at a dilution of 1 : 1000 was higher than that of the reference negative at 1 : 10 dilution. A comparison of the

TABLE.—*A Comparison of Micro-ELISA and Haemagglutination Inhibition Tests for Rubella Antibody*

Haemagglutination inhibition titres	Micro-ELISA results: No. of sera negative (E400 ⩽ 0·02)	Micro-ELISA results: No. of sera positive (E400 > 0·2)	Micro-ELISA results: Mean ELISA value (E400)
⩽1/8	26	5	0·15
1/16 and 1/32	2	18	0·34
1/64 and 1/128	0	72	0·40
1/256 and 1/512	0	49	0·49
⩾1/1024	0	16	0·71

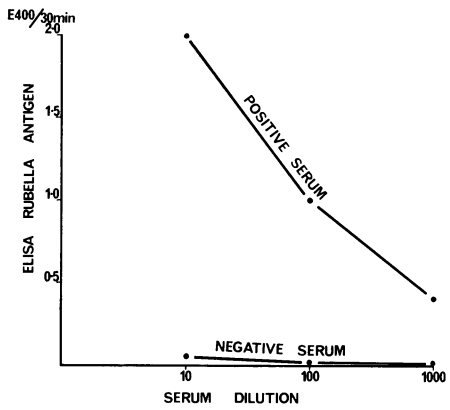

FIG.—The results of Rubella Micro-ELISA tests on the reference positive and negative sera at different dilutions.

HI and Micro-ELISA results on the routine batch of sera is given in the Table. Twenty-six of the 31 sera with HI titres of 1 : 8 or less gave negative ELISA readings. Of the 157 sera with HI titres of 1 : 16 or higher, 155 were positive by ELISA (a total discrepancy rate of 3·7% between ELISA and HI). The mean ELISA values of each group correlate with the HI titres.

DISCUSSION

Although these are only preliminary studies, they indicate that the Micro-ELISA can efficiently detect rubella antibody. The positive and negative reference sera showed, as expected, clear differences in ELISA values; however the "mock" routine assay on the batch of 188 sera also gave very encouraging results. It should be remembered that these sera were not absorbed or pretreated in any way and they were tested at a single high dilution. Even so, the ELISA results on these showed a 96% correspondence rate with the haemagglutination inhibition tests.

There is an obvious positive correlation between the ELISA values and HI titres but to establish the statistical basis of this relationship will require many more results from comparative tests.

The Micro-ELISA is simple, economical and well suited for use in the routine laboratory. In this context it is particularly valuable to have objective results such as those which can be obtained in this test using only a simple inexpensive spectrophotometer. It is likely that plates presensitized with the antigen, and the appropriate enzyme labelled antiglobulins, will soon become available from commercial sources. When class specific anti-immunoglobulin conjugates are employed it is possible to measure the IgG and IgM Rubella antibody levels separately by ELISA.

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