Enzyme-linked immunosorbent assay for the diagnosis of recent rubella infection

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SUMMARY This paper presents further evidence of the sensitivity and reproducibility of ELISA for the detection of rubella antibody. This test can also be extended to include the diagnosis of recent rubella infections once the index of significance has been computed based on the estimated ELISA ratio of absorbance between the acute and convalescent serum samples. There is a greater than 90% agreement when compared to the results obtained by the haemagglutination-inhibition test. It should be further evaluated for rubella using purified rubella antigen to increase sensitivity and reproducibility.

The enzyme-linked immunosorbent assay (ELISA) was developed by Engvall and Perlmann¹ and a micromodification of this assay for the detection of rubella antibodies was introduced by Voller and Bidwell.² Many papers have since been published on the use of ELISA for the detection of rubella antibodies.³⁻⁶ This technique appears to offer advantages over other serological techniques and this paper reports a comparative study of human sera for rubella antibodies using this assay and the haemagglutination-inhibition test. We also present an analysis of results based on 99 paired serum samples which have been submitted to our laboratory for the diagnosis of recent rubella infection.

Material and methods

The ELISA tests were carried out on Cooke microtitre 96 well polystyrene plates (Microelisa, Dynatech Laboratories Ltd) sensitised with a 1/100 dilution of a commercially available rubella-haemagglutinating antigen (Burroughs Wellcome) diluted in 0.05 mol/l carbonate buffer, pH 9.6. To each well of the plate was added 200 μ l of the diluted antigen solution and the plates incubated for two hours at room temperature in a humidified chamber. The plates were then washed three times in phosphate-buffered saline containing 0.05% Tween 20 (PBS-Tween), allowing three minutes between each wash. The plates were then shaken dry and used immediately.

A pooled human serum sample with a haemagglutination-inhibition (HI) titre of 1/320 was used as the reference positive serum and a pooled human serum sample with no detectable antibodies to rubella—that is, HI titre < 1/10—was used as the reference negative serum. These sera, like all the test samples, were used at 1/100 dilution as determined by checkerboard titration as being optimal under the conditions of the test.

Three hundred and sixty-seven single serum samples and 99 paired serum samples were tested for rubella antibodies by the HI test⁷ and retested by the micro-ELISA procedure similar to that described by Voller *et al.*² Amounts (200 μ l) of 1/100 dilution of serum samples were added to duplicate wells of the sensitised plates and these were incubated for two hours at room temperature. The plates were then shaken dry and washed in three changes of PBS-Tween. They were again shaken dry and to each well was added 200 μ l of a 1/700 dilution of alkaline phosphatase conjugated antihuman globulin prepared in our own laboratory. After overnight incubation at 4°C, the plates were emptied and washed as before. A 200 μ l amount of the substrate (*p*-nitrophenyl phosphate 1 mg/ml in 10% diethanolamine buffer, pH 9.8 with 0.5 mmol/l MgCl₂) was then added to each well. After 60 to 90 min, the reaction was stopped by the addition of 50 μ l of 3 mol/l NaOH to each well. The time of stopping the reaction was based on the reference positive serum sample giving a reading of about 1.0 using a spectrophotometer to measure absorbance at 405 nm.

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Results

DETERMINATION OF THE ABSORBANCE CUT-OFF VALUE

Of the 367 sera examined, 131 were found to be negative with HI titre of less than 1/10. These were analysed by ELISA and a mean absorbance reading of 0.16 was obtained. The standard deviation gave a value of 0.087. In order to obtain an absorbance cut-off value at 95% confidence interval, the standard deviation was doubled and then added to the mean value. This cut-off value was 0.33 which is about twice the mean absorbance value. Any serum sample which gave an absorbance reading of greater than or equal to 0.33 was considered to be positive for rubella antibody. Using this value, it was found that of the 131 HI negative sera, five samples were found to be positive by ELISA. Of the 236 sera which were HI positive, 223 were positive by ELISA. If the total number of specimens were taken together, 349 out of 367 agreed giving a concordant result of 95.1%.

DETERMINATION OF CONFIDENCE INTERVAL BETWEEN ABSORBANCE VALUES OF DUPLICATE WELLS

By the method of paired-comparison based on readings in duplicate wells on a random sample of

80 specimens, a confidence interval of 0.07 was obtained. From this, it was deduced that the maximum permissible variation of absorbance of duplicate wells for each serum was 0.07. Any variation greater than this value between duplicate wells required the assay to be repeated.

REPRODUCIBILITY OF ELISA

Fifteen serum samples with HI titres of 1/80 were analysed in duplicates by ELISA to study the effect of day to day variations. The results (Table 1) indicated that ELISA readings were highly reproducible. Variations in absorbance readings between duplicate wells were almost always less than the confidence interval of 0.07. The same was also observed for the reference positive and reference negative serum samples.

DETERMINATION OF AN ELISA INDEX OF SIGNIFICANCE FOR THE DIAGNOSIS OF RECENT RUBELLA INFECTION

For this purpose a comparison of the ratio of convalescent antibody level over acute antibody level by HI and ELISA was made using 99 paired serum samples. The ratio was used instead of other informative quantities because the increase in HI antibody titre between acute and convalescent serum samples would then be "location-free"—that is, a HI antibody titre increase from 1/10 to 1/40 is taken to be equivalent to an increase from 1/40 to 1/160.

Table 2 represents a comparison of the HI ratio and the estimated ELISA absorbance ratio based on the number of paired specimens in each group. Out of the 54 paired serum samples which showed no increase in HI titre between the acute and convalescent sera—that is, HI ratio of 1, the estimated ELISA absorbance ratio was 1.19. When the HI ratio was 4 indicating a significant fourfold increase in titre between the paired sera, the estimated ELISA absorbance ratio was 1.80. The ELISA ratio increased consistently with corresponding increase in the HI ratio. From the estimated ELISA ratios, it was calculated that an ELISA ratio of 1.80 and greater should be considered as the index of significance when used in the diagnosis of recent rubella infections. This value was computed to give

Table 1 Day to day variation of ELISA absorbance values

Serum samples	Absorbance values Day 1		Day 2		Mean
	Duplicate	Mean	Duplicate	Mean	
1	0.88 0.77	0.83	0.88 0.86	0.87	0.87
2	0.83 0.80	0.82	0.83 0.80	0.82	0.82
3	0.89 0.80	0.85	0.87 0.85	0.86	0.86
4	0.85 0.78	0.82	0.88 0.84	0.86	0.86
5	0.82 0.77	0.85	0.88 0.81	0.85	0.85
6	0.70 0.73	0.72	0.71 0.78	0.75	0.75
7	0.76 0.80	0.78	0.83 0.80	0.82	0.82
8	0.86 0.82	0.84	0.95 0.94	0.95	0.95
9	0.73 0.73	0.73	0.80 0.79	0.80	0.80
10	0.40 0.40	0.40	0.44 0.42	0.43	0.43
11	0.73 0.79	0.76	0.74 0.76	0.75	0.75
12	0.99 1.04	1.02	0.96 0.97	0.97	0.97
13	0.88 0.84	0.86	0.83 0.82	0.83	0.83
14	0.84 0.81	0.83	0.86 0.85	0.86	0.86
15	0.40 0.35	0.38	0.39 0.35	0.37	0.37
Reference + ve	0.90 0.96	0.93	0.81 0.92	0.87	0.87
Reference - ve	0.35 0.30	0.32	0.32 0.25	0.28	0.28

Table 2 Comparison of HI ratios and ELISA absorbance ratios

	1	2	4	8	16	32	64	128
*HI ratio	1	2	4	8	16	32	64	128
†Estimated ELISA ratio	1.19	1.40	1.80	2.40	3.11	3.30	3.48	3.54
No of paired samples analysed	54	1	2	1	10	17	9	5

$$* \text{HI ratio} = \frac{\text{HI titre of convalescent serum}}{\text{HI titre of acute serum}}$$

$$\dagger \text{Estimated ELISA ratio} = \frac{\text{Absorbance value of convalescent serum per group}}{\text{Absorbance value of acute serum per group}}$$

confidence interval of greater than 95%.

In the analysis of the 99 paired serum samples, it was found that 51 of 55 pairs (92.7%) which had a HI ratio of less than four had an estimated ELISA absorbance ratio of less than 1.80. Of the 44 positive paired serum samples, 41 (93.2%) gave an estimated ELISA absorbance ratio of ≥ 1.80 . Overall, 92 of the 99 paired serum samples were in total agreement by comparison of HI ratio and estimated ELISA absorbance ratio, giving a concordant result of 92.9%.

Discussion

The application of the ELISA technique in the serological diagnosis of viral infection is still in its infancy and before it can be widely adopted as a routine serological tool, it should be extensively and thoroughly evaluated by comparing it with other standard procedures. In this paper, we have presented results which showed that ELISA for the detection of rubella antibodies is sensitive and reproducible. The test can be made even more sensitive by reducing the cut-off value if purified antigen such as the preparation used by Vejtorp⁸ had been used. This will lessen the discrepancy rate between the HI and ELISA results in this project.

An important finding from this study is that the ELISA technique can be used not only to determine the immune status of an individual by testing single specimen of serum, but can also be used as a serological diagnosis of recent rubella infection by determining the ratio of ELISA absorbance between acute and convalescent sera. The index of significance was determined as 1.80 in this study, a value which

gives a greater than 95% confidence. Our preliminary study is encouraging and should be further evaluated for rubella as well as for other viral infections.

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References

- ¹ Engvall E, Perlmann P. Enzyme-linked immunosorbent assay (ELISA). Quantitative assay of immunoglobulin G. *Immunochemistry* 1971;**8**:871-4.
- ² Voller A, Bidwell DE. A simple method for detecting antibodies to rubella. *Br J Exp Pathol* 1975;**56**:338-9.
- ³ Bidwell DE, Bartlett A, Voller A. Enzyme immunoassays for viral diseases. *J Infect Dis* 1977;**156**:274-8.
- ⁴ Morgan-Capner P, et al. A comparison of three tests for rubella antibody screening. *J Clin Pathol* 1979;**32**:542-5.
- ⁵ Leinikki PO, et al. Enzyme-linked immunosorbent assay determination of specific rubella antibody levels in micrograms of immunoglobulin G per millilitre of serum in clinical samples. *J Clin Microbiol* 1978;**8**:418-23.
- ⁶ Forghani B, Schmidt NJ. Antigen requirements, sensitivity, and specificity of enzyme immunoassays for measles and rubella viral antibodies. *J Clin Microbiol* 1979;**9**:657-64.
- ⁷ Lam SK. The seroepidemiology of rubella in Kuala Lumpur, West Malaysia. *Bull WHO* 1972;**42**:127-9.
- ⁸ Vejtorp M. Enzyme-linked immunosorbent assay for determination of rubella IgG antibodies. *Acta Pathol Microbiol Scand* 1978;**86**:387-92.

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