

## EDITORIAL

### Rubella antibody screening

During the last three years a series of articles has been published in the Journal from a variety of sources concerned with aspects of rubella antibody screening. Continued development in this field has been stimulated by recommendations for more extensive screening of women prior to immunization with rubella vaccine, a desire to achieve greater certainty in the distinction between those who require immunization and those who do not and attempts to overcome the known deficiencies of existing tests. It now seems clear that a new generation of screening tests is becoming established in clinical virology laboratories and it is interesting to review their emergence and relative merits.

Following the isolation of rubella virus in the early 1960s descriptions of a neutralization test, a complement fixation test and an immunofluorescence test for rubella antibody appeared. However, it was the rubella haemagglutination-inhibition (HI) test, first described by Stewart *et al.* (1967) which provided a relatively simple and accurate method for the serological diagnosis of rubella and assessment of immune status. The use of the rubella HI test increased rapidly and it has been the cornerstone of rubella serology during the 1970s. By contrast the other tests retained only a very limited application.

There is no doubt that the rubella HI test has an appropriate sensitivity for a rubella antibody screening test, and the presence or absence of rubella HI antibody correlates well with immunity or susceptibility to primary rubella virus infection. However, all sera contain non-antibody inhibitors of rubella haemagglutinin. These are predominantly lipoproteins and the most potent inhibitors are the low-density lipoproteins. Accordingly all sera have to be pre-treated prior to screening for rubella antibody in order to remove these inhibitors. Absorption with kaolin or precipitation with manganous chloride/heparin mixtures have been the most widely used methods for removing non-antibody inhibitors. In the June 1981 issue Traavik, Spanne & Mennen published further investigations into the extent of the removal of non-antibody inhibitors by various pre-treatment procedures. They showed that use of polyvalent anion-divalent cation combinations results in the presence of residual non-antibody inhibitors in the majority of sera and in considerable amounts in some of them. The work was centred on an evaluation of Aerosil, a colloidal, pyrogenic silica, for the pre-treatment of sera. They concluded that Aerosil was the most effective treatment but offered few advantages for those already using kaolin. However, there is no doubt that kaolin fails to remove non-antibody inhibitors completely on some occasions. During a P.H.L.S. collaborative survey to derive a minimum immune titre (MIT) of rubella HI antibody Bradstreet *et al.* (1978) found that all sera with 24 or more i.u./ml of HI antibody

were positive by IF, radial haemolysis and by HI after flotation centrifugation which separates antibody from lipoproteins with certainty. However, only 9 of the 12 sera tested with 12 i.u./ml of HI antibody had detectable IF antibody and only 10 were positive after flotation centrifugation. Thus rubella HI activity equivalent to 12 i.u./ml of HI may represent residual non-specific inhibitors rather than specific antibody. The authors concluded that the MIT should be equivalent to 24 i.u./ml rubella HI antibody and that persons with HI activity below this titre should be immunized. In the light of further experience this MIT was reduced to 15 i.u./ml. Use of such an MIT in interpreting the results of rubella HI tests avoids the majority of errors in reporting that would arise from residual non-antibody inhibitors. Nevertheless the HI test remains a time-consuming, multi-step procedure which is not particularly appropriate for the routine screening of large numbers of sera.

An entirely different approach was provided by the emergence of radial haemolysis (RH) as a method of detecting antibody to haemagglutinating viruses. Detection of rubella antibody by RH was first described by Skaug, Østravik & Ulstrup (1975), but the report of a P.H.L.S. working party in this journal (Kurtz *et al.* 1980) provided a simple, economical and robust method. Particular features of this test include the use of sheep red cells, the incorporation of Richardson's preserved complement into the gel and the use of square Petri-dishes allowing 60 test sera to be screened in one gel.

The only pre-treatment of test sera required is heat inactivation at 60 °C for 20 min. Wells cut in the gel are then filled with serum, and after overnight incubation the zones of haemolysis are read by trans-illuminating against a black background. The test was shown to be sensitive enough to detect 5.6 i.u./ml rubella antibody, and five laboratories collaborated in testing more than 8000 sera. A reference positive serum containing 15 i.u./ml was used and 88.4 % of test sera gave a zone equal to or greater than that given by this standard. No zone was seen with 8.9 % of sera, and this indicates susceptibility to rubella infection. All sera were also tested in a control gel which does not contain viral antigen, and non-specific haemolysis interfered with test readings in only 0.4 %. Finally small zones less than those found with the 15 i.u./ml standard were given by 2.2 % of sera. As many as possible of the negative sera and those giving small zones were tested by HI and only fourteen discrepancies were found. Eight were HI positive/RH negative and, of these, two were specific IgM-containing sera (this class of antibody often being unreactive in RH) and the other six contained no IF antibody. The remaining discrepancies were HI negative/RH positive, and all these contained IF antibody. Thus in over 8000 sera there was a slight gain in sensitivity with RH and no false positive results. The authors therefore concluded that RH should replace HI for rubella antibody screening. This recommendation has certainly been adopted in the U.K., with consequent improvement in the ease with which large numbers of sera can be screened and a greater certainty in the interpretation of results in relation to immunity and susceptibility. Nevertheless, there is still caution about the interpretation of zones of haemolysis smaller than the 15 i.u./ml standard and these are usually reported as follows. 'Low level of rubella antibody (< 15 i.u./ml).

Regard as non-immune.' This seems a pity since the 15 i.u./ml MIT was chosen to compensate for a deficiency of the HI test. Rubella HI activity equivalent to less than 15 i.u./ml could be due to residual non-antibody inhibitors. However, it may be due to specific rubella antibody and there is no evidence that such low levels of antibody are not protective. Non-antibody inhibitors do not interfere with the RH test at all, and if screening is performed by this method it may not be necessary to use such a high MIT. Mortimer *et al.* (1981) addressed themselves to this problem and asked the question, 'Are many women vaccinated against rubella unnecessarily?'. They studied 1317 women attending a single general practice. Of the 141 (10.7%) reported as requiring immunization 43% had low levels of rubella antibody (< 15 i.u./ml) and the remainder had no detectable rubella antibody by RH. Thirty-three women in each group were studied in more detail and post-immunization specimens were tested for rubella-specific IgM. The principle behind the investigation was that those women who were susceptible to rubella would react to immunization with a primary response which would include the production of rubella-specific IgM. By contrast those with pre-existing antibody might produce a secondary response with a significant increase in antibody, but that specific IgM would not be a component of this increase. The results showed that specific IgM was not detectable in any of the post-immunization specimens from the 33 women with low levels of antibody by RH in the pre-immunization specimen. On the other hand specific IgM was present in the post-immunization specimens of 29 of the women who had no rubella antibody in the pre-immunization sample; the result was equivocal in 2 and definitely negative in 2.

Thus there is no doubt that a significant proportion of women currently reported as in need of rubella immunization are in fact immune and that a 15 i.u./ml MIT seems inappropriate for rubella antibody screening by RH. The results of Mortimer *et al.* (1981) suggest that this could be lowered to 5 i.u./ml. Two questions remain: first, is there proof that such low levels of antibody protect against foetal infection on re-exposure and second, is there any rubella antibody screening test which is suitable for the detection of such low levels of rubella antibody in routine practice?

The answer to the first question is provided by Cradock-Watson and his colleagues, publishing in the October 1981 issue of the *Journal*. Significant rises of rubella antibody sometimes occur in the absence of any symptoms. If the increase is of specific IgG only this is regarded as re-infection, whereas a specific IgM response indicates primary subclinical rubella. Cradock-Watson *et al.* (1981) studied 40 cases of symptomless infection during pregnancy. Thirty-four of these were re-infections and in nine of these the pre-existing antibody was less than 15 i.u./ml. These 34 re-infections were compared with six subclinical primary infections. Possible intrauterine infection was investigated by testing a serum from the baby shortly after birth for specific IgM and by testing a specimen taken after the age of eight months for specific IgG. No evidence of intrauterine infection was found in 33 of the 34 babies born to mothers re-infected during pregnancy and in the other case the explanation of persistent IgG antibody at 11 months was almost certainly acquired rubella at 5½ months of age. By contrast three of the six infants

born to mothers who had primary subclinical rubella during pregnancy showed evidence of intrauterine infection and one had clinical evidence of congenital rubella. This is by far the largest and most comprehensive study of rubella re-infections during pregnancy. The results indicate that any amount of pre-existing rubella antibody is likely to protect against intrauterine infection on re-exposure to rubella, although the authors quote the three instances in the literature in which apparent re-infection in the mother resulted in foetal infection. It should also be noted that the distinction between re-infection and primary subclinical infection may become more difficult in the future. Thirty-three of the 34 cases of re-infection described by Cradock-Watson *et al.* (1981) had pre-existing antibody as a consequence of natural infection and in none of these was IgM detectable in the serum taken after re-infection. However, a trace of IgM was detected after re-infection in the one case whose pre-existing antibody was vaccine induced. In the future when more women have vaccine-induced immunity the detection of specific IgM may not provide a reliable distinction between re-infection and primary subclinical infection. Moreover, it remains to be seen whether low levels of antibody induced by vaccine are as protective against foetal infection as those persisting after natural infection.

Finally, the question of whether existing screening methods are sufficiently sensitive and reliable for the detection of low levels of antibody in routine practice remains unresolved. Certainly the results of Mortimer *et al.* (1981) indicate that this can be achieved using RH in a limited, special study. Nevertheless, small zones of 4–6 mm diameter produced by specific antibody are not always easy to recognize around wells 2–3 mm in diameter, particularly if there is some reaction in the control plate. The authors suggest that increasing the sensitivity by using larger well sizes and a lower concentration of red cells should be further investigated. They also compared the results of testing for rubella antibody by HI and ELISA with those obtained using RH. None of the individuals whose pre-immunization serum was reactive in the HI test gave a positive result for specific IgM antibody in the post-immunization specimen. Nevertheless, the HI test cannot be used on a large scale with an MIT of less than 15 i.u./ml for the reasons stated above. With a commercially available ELISA test (Rubazyme, Abbott) all the pre-immunization sera which were positive were associated with a negative result for specific IgM in the post-immunization specimen. By contrast specific IgM was found in the post-immunization specimen in 29 of 35 individuals whose pre-immunization specimens were unreactive by ELISA. Thus it seems that the ELISA test has an appropriate sensitivity and specificity for the detection of rubella antibody below 15 i.u./ml. Nevertheless, much more experience with weakly reactive sera in this test is required before recommendations can be made concerning its widespread use.

In conclusion there seems little justification for continuing to use the HI test for rubella antibody screening. At present there is sufficient experience with RH to use this test as a replacement. Equally it is clear that the use of a 15 i.u./ml MIT, which was an appropriate compensation for a deficiency in the HI test, is resulting in the unnecessary immunization of a significant proportion of women.

If the results of Mortimer *et al.* (1981) are widely applicable then only 60% of women with less than 15 i.u./ml rubella antibody are truly sero-negative and in need of immunization. Many of the other 40% could be screened out using the RH test with a lower MIT. An ELISA test may also prove to be an appropriate method for screening, but this requires further evaluation.

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