Evaluation of a commercial antibody capture enzyme immunoassay for the detection of rubella specific IgM

J HODGSON,* P MORGAN-CAPNER
From the Department of Medical Microbiology, King's College Hospital, Denmark Hill, London SE5 9RS

SUMMARY A commercial M antibody capture ELISA kit (Rubenz M) for the detection of rubella specific IgM was evaluated in comparison with M antibody capture radioimmunoassay. A total of 248 sera were evaluated, including sera from cases of primary postnatal rubella, congenital rubella, infectious mononucleosis, and sera which contained rheumatoid factor. No false positive results were obtained but two high positive sera gave Ruben M values which were below the value recommended as indicative of a positive result. We therefore propose changes in the criteria used for assessing the significance of the results obtained. These changes improve the accuracy of the assay without loss of specificity.

The ability to detect rubella specific IgM is an essential requirement for the serological diagnosis of primary postnatal rubella as sera are often received too late in the evolution of the illness for diagnostic rises in total antibody titre or rubella specific IgG to be shown. Even when diagnostic rises in titre are shown, the demonstration of appreciable concentrations of rubella specific IgM indicates primary rubella rather than reinfection. This latter situation is particularly applicable to subclinical infections as a reinfection appears to be of minimal risk to the fetus.1 Furthermore, the detection of rubella specific IgM in neonatal sera has become an established method of diagnosing congenital rubella.

Several techniques are currently available for the detection of rubella specific IgM. Sera may be fractionated by sucrose density gradient ultracentrifugation2 or by gel filtration,3 with haemagglutination inhibition or immunofluorescent detection of rubella specific antibodies being performed on the fractions containing immunoglobulin. These techniques are time consuming, only limited numbers of sera may be evaluated, and technical problems occur such as the presence of residual non-specific inhibitors of haemagglutination. These assays are now being replaced by immunological assays that do not require physical separation of IgM from IgG and IgA.

One variety of such assays is based on the binding of rubella specific IgM from patients' sera to rubella antigen adsorbed to a solid phase. The bound rubella specific IgM is detected by incubation with antihuman antibody labelled radioactively4 or with an enzyme.5 Such assays, however, usually require purified rubella antigen for evaluation of adult sera, although crude rubella antigen is satisfactory for assays performed on neonatal sera.6 In addition, assays of this type are liable to give false positive reactions with sera containing rubella specific IgG and rheumatoid factor.7 A commercially available enzyme immunoassay for rubella specific IgM (Rubazyme M, Abbott Diagnostics Division, Basingstoke, UK) appears to have overcome the problem of rheumatoid factor and has been evaluated.8

A further variety of assays are the IgM antibody capture assays, in which the solid phase is coated with antihuman IgM. Incubation with a dilution of the patient's serum results in the selective binding of patient's IgM. Any of the bound IgM specific for rubella is detected by incubation with rubella antigen followed by incubation with labelled antirubella antibodies or agglutinable red blood cells. The assay has been described with a rabbit antirubella antibody labelled with 125I (MACRIA),9 which has since been modified by the use of a 125I-labelled monoclonal antirubella antibody.10 Many authors have

*Seconded from Dulwich Public Health Laboratory, Dulwich Hospital, East Dulwich Grove, London SE22 8QF.

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Results of testing for the presence of rubella specific IgM by MACRIA and by Rubenz M

<table>
<thead>
<tr>
<th>Sera</th>
<th>Total</th>
<th>Criteria recommended by Northumbria Biologicals Ltd</th>
<th>Altered criteria using corrected absorbance value compared with 5 au control (%)</th>
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<tr>
<td></td>
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<td>Rubenz M positive</td>
<td>Rubenz M negative</td>
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<td>Remote rubella MACRIA negative</td>
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<tr>
<td>Remote rubella MACRIA negative (&lt;1 au)</td>
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<td>Recent rubella MACRIA low positive (&gt;10 au)</td>
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</tr>
<tr>
<td>Recent rubella MACRIA (1-0-3.3 au)</td>
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<td>Sera containing rheumatoid factor</td>
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<td>12</td>
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<tr>
<td>Sera from cases of infectious mononucleosis</td>
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<td>10</td>
</tr>
<tr>
<td>Total</td>
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au = arbitrary unit; IU = international unit; HI = haemagglutination inhibition.

reported an IgM antibody capture assay using an enzyme linked indicator system. The sensitivity of this method was similar to that obtained with MACRIA but false positive results have been obtained with a polyclonal antirubella owing to the presence of rheumatoid factor, a problem not reported with MACRIA. More recently, the problem of rheumatoid factor in the IgM antibody capture ELISA technique appears to have been overcome by the use of a F (ab')2 conjugate. Many variations on the technique using red blood cells as an indicator have been described. This method, although the least expensive, is more laborious in that a prior dilution series of patient's serum may have to be performed whereas in assays using labelled antirubella antibody only a single dilution of serum is used.

The use of an 125I label may impose considerable difficulties in many laboratories not equipped to deal with radioactivity and the label has a limited life of only a few months. The use of an antirubella indicator labelled with an enzyme overcomes these difficulties and allows such an assay to be widely available.

We report our evaluation of the first commercially available rubella specific IgM assay (Rubenz M, Northumbria Biologicals Ltd, Cramlington, UK) of the antibody capture type, which uses an enzyme labelled monoclonal antirubella antibody.

Material and methods

SERA

All sera previously shown to contain rubella specific IgM had been stored at 4°C with added azide. The remaining sera had been stored at −20°C. One hundred sera from patients with primary postnatal rubella were examined. Of these, 87 sera were considered to be high positive and 13 low positive by MACRIA (Table). A further 12 sera had given an equivocal result by MACRIA. One hundred and two sera were tested from patients with remote primary rubella who gave a history of rubella like symptoms or of recent contact with a rubelliform illness. Rubella specific IgM was not detected by MACRIA in these sera, of which 11 were especially selected as they had raised haemagglutination inhibition titres of ≥800 IU.

Twelve sera were available from infants with congenital rubella. Eight were positive by MACRIA, two gave equivocal values (collected at 4 months and 1 year of age), and two were negative (collected at 9 months and 1 year of age).

To determine the possible effect of rheumatoid factor, 12 sera were tested which contained high concentrations of rheumatoid factor by latex agglutination and rubella specific IgG detected by radial haemolysis. Ten sera were tested from patients with infectious mononucleosis. Seven of
these sera had previously been shown to be reactive in MACRIA.²⁰

MACRIA
The MACRIA method used was adapted from that described by Mortimer et al.⁹ Polystyrene beads, 6-4 mm diameter, made by the Precision Plastic Ball Co, Chicago, USA and supplied by Northumbria Biologicals Ltd, UK, were used as the solid phase. The beads were coated with rabbit antihuman IgM (Dako, Copenhagen, Denmark, supplied by Mercia Broacades Ltd, UK) by agitating in a 1/500 dilution in N-HCl for 1 h at room temperature. The beads were stored at 4°C for at least 48 h before use. For use, the required number of beads were washed once with phosphate buffered saline (PBS) and then incubated for 3 h at room temperature in PBS containing 1% bovine serum albumin. Test and control sera (5 μl) were diluted 1/40 in PBS containing 0.05% Tween 20 (PBST) in the wells of polystyrene plates (Abbott Ltd, Basingstoke, UK) and an unwashed coated bead added to each well. The trays were incubated at 37°C for 3 h before washing each bead three times in PBST. Judith strain rubella haemagglutinin, supplied by the Public Health Laboratory Service, Colindale, UK, was diluted 1/10 in PBST, 200 μl added to each bead, and incubated for 18 h at 4°C. The beads were washed three times in PBST. To each bead was added 200 μl of ¹²⁵I-labelled mouse IgG antirubella monoclonal antibody (kindly supplied by Dr R Tedder, Middlesex Hospital Medical School, UK), diluted in PBS containing 0.2% Tween 20, 10% heat inactivated human rubella antibody negative serum, and 20% heat inactivated rabbit serum so that 200 μl gave a count of 50 000/min. After incubation at 37°C for 3 h the beads were washed four times in PBST and the bound radioactivity determined by counting each bead for 100 s in a 16 channel gamma counter (NE 1600, Nuclear Enterprises, Edinburgh, UK).

A negative control serum and a series of positive control sera containing 40, 10, 3-3, and 1-0 arbitrary units (au) of rubella specific IgM were included in each assay. A calibration curve was obtained by plotting the unitage of the control sera against the counts/100 s obtained for each. The results of the test sera were expressed as au of rubella specific IgM by comparison with the standard calibration curve. Sera were considered high positives if they contained \( \geq 10-0 \) au of rubella specific IgM, low positives at values of \( >3-3-9-9 \) au, and equivocal at values of \( 1-0-3-3 \) au. Sera at values \( <1-0 \) au were considered negative.

RUBENZ M
The assay was performed as indicated by the manufacturers. Polystyrene wells coated with antihuman IgM were supplied in strips of 12 wells. One or more strips could be used depending on the number of sera to be assayed. The strips were conveniently located in a frame supplied with each kit. Briefly, 100 μl of a 1/50 dilution of serum in PBST were incubated in duplicate coated wells for 4 h at 37°C. After washing, to one of each pair of wells was added 100 μl of PBST (the control well) and to the other was added 100 μl of Judith strain rubella antigen (the test well). The strips of wells were left overnight in a humid atmosphere at room temperature. Next morning the wells were washed and 100 μl of a monoclonal antirubella antibody conjugated to horseradish peroxidase added to each well. After incubation for 3 h at 37°C and subsequent washing, 100 μl of substrate, orthophenylenediamine in phosphate/citrate buffer with H₂O₂, were added. After leaving at 37°C for 20 to 30 minutes, the reaction was stopped by the addition of 100 μl of 5N H₂SO₄ to all wells. The absorbance of each well was read in a Dynatech MR 580 Microelisa autoreader. Each assay incorporated a high titre positive (40 au), a low titre positive (5 au), which had been standardised by MACRIA, and a negative control serum. For every serum tested, the absorbance of the 'control' well was subtracted from that of the 'test' well to give a corrected absorbance value (CAV). Any serum which gave a CAV equal to or greater than the CAV of the 5 au control serum (the CAV₅) was considered positive. In addition, we calculated the CAV of the test serum as a percentage of the CAV₅.

Results
The assay was easy to perform and fitted well into the normal working day. No reactivity sufficient to influence the categorisation of a serum was seen in the control wells.

The 102 sera from cases of remote rubella were negative by Rubenz M (Table) and all gave a CAV less than half of that of the CAV₅. Of the 87 sera from cases of recent primary rubella which had given a high positive MACRIA result, however, two were negative by Rubenz M using the advised criteria. The CAVs for these two sera were 86% and 82% of the CAV₅. Only three of the low positive MACRIA sera gave positive results by Rubenz M when the advised criteria were used, but a further four gave CAVs >80% of the CAV₅, and three gave CAVs 50-80% of the CAV₅.

One of the 12 sera equivocal by MACRIA was positive by Rubenz M, with a further nine giving
CAVs between 50 and 80% of the CAV.<sub>3</sub> The remaining two sera gave CAVs of <50% of the CAV.<sub>3</sub>

The two sera from cases of congenital rubella which were negative by MACRIA were negative by Rubenz M. Six of the seven strong positive MACRIA sera were positive, with the remaining serum giving a CAV 97% of the CAV.<sub>3</sub>. The one MACRIA low positive serum gave a CAV 85% of the CAV.<sub>3</sub>. The two sera from cases of congenital rubella which had given an equivocal result by MACRIA gave, in one case, a CAV of <50% of the CAV.<sub>3</sub> and in the other a CAV 51% of the CAV.<sub>3</sub>

All the 12 sera containing rheumatoid factor and rubella specific IgG were negative by Rubenz M, giving CAVs <50% of the CAV.<sub>3</sub>. Nine of the 10 sera from cases of infectious mononucleosis gave similar negative results. The remaining serum had given a MACRIA result of 20 au, but in Rubenz M gave a CAV which was 59% of the CAV.<sub>3</sub>

Discussion

The Rubenz M assay was convenient and easy to use with the particular advantage of the solid phase comprising polystyrene wells in strips of 12, so enabling a few sera to be tested without wasting expensive materials. The kits are stated to be usable for up to three months and, although no formal shelf life study was performed, the kit worked satisfactorily for at least one month. Results of the assay could be assessed with the unaided eye by comparing test wells with the positive and negative sera control wells. We would, however, recommend spectrophotometric reading of the results to avoid possibly missing low positive results. We did not see false positive results due to bound rheumatoid factor reacting directly with the conjugated antirubella as has been found by others. As no appreciable reactivity was seen with the control wells, it would not appear necessary to perform such controls. Indeed, they are not performed with the MACRIA technique. It may be wise, however, to evaluate each serum in duplicate test wells as this has been shown to minimise any effects of well-to-well variation (personal observation) and would reduce the chance of technical and mechanical error. Such recommendations are being considered by the manufacturers.

The technique of MACRIA for the detection of rubella specific IgM is used routinely in our laboratory and is now well established. It does have a potential sensitivity greater than any other current assay, but the usable sensitivity is limited by values of reactivity of 1-0-3-3 au occurring with sera in which there is usually no supporting evidence for recent primary postnatal or congenital rubella.

Indeed, occasional sera have been evaluated in which values of 3-3-10-0 au have not been considered to indicate recent primary or congenital rubella.

MACRIA and Rubenz M are similar in many respects with the only important differences being the nature of the solid phase and the labelling of the monoclonal antirubella with 125I in place of peroxidase. It might therefore be expected that a good correlation would be observed between the results of the two assays. Although there was a good correlation for the presence of rubella specific IgM reactivity, a lack of correlation in the degree of reactivity was seen. Such a lack of correlation between the quantitative results of different assays for the detection of rubella specific IgM is not infrequent (personal observation).

No false positive results were seen when using the criteria recommended by Northumbria Biologicals. Such criteria however, resulted in two strong positive sera being considered negative. These sera had been collected 11 and 24 days after the onset of the rubella rash, were the second sera of a pair showing rubella haemagglutination inhibition seroconversion and had given MACRIA results of 14 and 22 au respectively. They did, however, give CAV values >80% of the CAV.<sub>3</sub>. It is of interest that the latter serum was one of two sera strongly positive by MACRIA which had originally given completely negative results during the initial development of the Rubenz M assay. At this early stage the rubella haemagglutinin antigen used was produced from an RA27/3 strain of rubella virus. On using the Judith strain, both sera became reactive in Rubenz M so necessitating a change in the rubella haemagglutinin antigen used in the assay.

If the CAV value taken to indicate positivity was reduced to 80% CAV.<sub>3</sub>, a further four of the low positive sera, one of the congenital rubella high positive sera, and the congenital rubella low positive serum became positive. As all 102 negative sera and the 12 sera containing rheumatoid factor gave CAVs less than half of the CAV.<sub>3</sub>, a CAV in the range 50-80% could be considered equivocal and interpreted appropriately. The equivocal range for Rubenz M therefore included three of the low positive sera and one of the congenital rubella sera which had given an equivocal MACRIA result. It would be unwise to reduce the value taken to indicate positivity to a CAV of half of the CAV.<sub>3</sub> as nine of the MACRIA equivocal sera gave values in the 50-80% range. All 12 of the MACRIA equivocal sera were initially reported as there being no evidence of recent primary rubella. On re-examining the clinical details of the one serum in this group that gave a positive Rubenz M result, the assay may
have been detecting a trace of rubella specific IgM in an asymptomatic reinfection as the serum had been taken about 10 days after a rubella contact, had rubella specific IgG detectable by radial haemolysis, and gave a MACRIA result of 2-9 au.

The three sera that remained negative by Rubenz M in the low positive MACRIA group were all acute sera with low or negative haemagglutination inhibition titre, having been collected 3, 4, and an unstated number of days after the onset of the rash. The MACRIA results were 3-6, 8-0, and 5-0 au respectively, with rubella specific IgG not being detected by radial haemolysis. Such a combination of clinical details and serology would necessitate a further serum which would have established the diagnosis. The one serum from a case of congenital rubella which was negative by Rubenz M, despite having given an equivocal result by MACRIA (2-5 au), was collected at 18 weeks of age and so the absence of detectable rubella specific IgM would not have excluded congenital rubella and prospective evaluation for the persistence of rubella specific IgG would have been required. It has previously been shown that MACRIA is slightly more sensitive than the enzyme linked equivalent assay for the detection of rubella specific IgM in congenital rubella and our results agree with this conclusion. Evaluation and analysis of a large number of neonatal and infant sera, however, may show that the value of reaction taken as positive for this group may be reduced even more.

A further difference between the results of the two assays for rubella specific IgM is displayed by testing sera taken from cases of infectious mononucleosis. Of 10 tested, none were positive by Rubenz M and only one (which gave a MACRIA result of 20 au) gave an equivocal result, although a further six had been shown to give MACRIA values of 3-3-10 au. As similar antihuman IgM, rubella haemagglutinin antigen, and monoclonal antirubella are used for both assays, there is no apparent explanation for these results.

In conclusion, Rubenz M would appear to be an ideal assay for the detection of rubella specific IgM for laboratories not equipped to perform MACRIA as, from our evaluation, it has appropriate sensitivity and specificity. As for all assays for the detection of rubella specific IgM, however, the final interpretation of the result obtained will depend on a critical appraisal of all clinical and serological data available and the above recommended criteria may need reassessment when considerable "in use" experience has been obtained.

References


Requests for reprints to: Dr P Morgan-Capner, Department of Medical Microbiology, King's College Hospital Medical School, Denmark Hill, London SE5 8RX, England.