Public Health Laboratory Service IgM antibody capture enzyme linked immunosorbent assay for detecting rubella specific IgM

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SUMMARY A total of 468 sera were selected for the evaluation of the Public Health Laboratory Service’s IgM antibody capture enzyme linked immunosorbent assay kit (MACELISA) for detecting rubella specific IgM. The results obtained were compared with those obtained by IgM antibody capture radioimmunoassay (MACRIA). Sera from patients with primary postnatal rubella, congenital rubella, remote rubella, infectious mononucleosis, and recent infection with other agents were included, in addition to sera taken after rubella immunisation and sera containing rheumatoid factor and rubella specific IgG antibody. The assay exhibited a similar ability and comparable specificity to MACRIA for detecting rubella specific IgM antibody. The Public Health Laboratory Service MACELISA can be recommended if, as for all assays that detect rubella specific IgM, all the available clinical and serological data are taken into account when the results are interpreted.

The detection of rubella specific IgM is of established importance in diagnosing primary postnatal and congenital rubella and distinguishing rubella reinfections from primary subclinical rubella. Numerous methods have been described for detecting rubella specific IgM,1 but the well established methods, based on fractionation of serum by sucrose density gradient centrifugation2 and gel filtration,3 are being replaced by solid phase immunoassays.

Two varieties of these assays are in use. The first type is the solid phase antigen assay in which a solid phase coated with rubella antigen is sequentially incubated with a dilution of the patient’s serum and labelled antihuman IgM. Purified rubella antigen and procedures to negate possible false positive results due to rheumatoid factor are required. Consequently, these assays are usually routinely performed only when supplied as a kit by a commercial manufacturer, for example, Rubazyme-M, Abbott Laboratories, United Kingdom.*

The other variety of immunoassay is the IgM antibody capture type, in which the solid phase is coated with antihuman IgM and sequentially incubated with a dilution of the patient’s serum, rubella antigen, and either agglutinable red cells (as in SPARC,5 (solid phase attachment of red cells)) or an antirubella antibody labelled with iodine-125 (IgM antibody capture radioimmunoassay, MACRIA),6 or an enzyme (IgM antibody capture enzyme linked immunosorbent assay, MACELISA).7-9 An important advantage of these assays is that they do not require purified rubella antigen and, in general, are resistant to false positive results due to rheumatoid factor. In addition, MACRIA has been greatly improved by replacement of the hyperimmune antirubella antibody originally used with a monoclonal antibody.10 This monoclonal antibody has also been labelled with peroxidase and used in a commercial MACELISA (Rubenz M, Northumbria Biologicals Ltd, United Kingdom).11

We now report the evaluation in two laboratories of a rubella MACELISA that also uses a monoclonal antibody labelled with peroxidase and that was developed for the Public Health Laboratory Service by the Division of Microbiological Reagents and Quality Control, Central Public Health Laboratory, Colindale, United Kingdom (test centre 2).

Material and methods

SERAS All sera had been previously tested by MACRIA at

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King's College Hospital, London (test centre 1), and had been stored at -20°C, or at 4°C with added azide if previously shown to contain rubella specific IgM. They were evaluated by the Public Health Laboratory Service MACELISA at test centre 1 and independently at test centre 2.

A total of 376 sera were evaluated from patients with recent primary postnatal rubella, congenital rubella, remote rubella, and rubella after immunisation. Tables 1–3 show the relevant details. A further 27 sera were selected that had given an equivocal result by MACRIA (Table 2). These comprised sera from adults and children who had recently had a rubelliform illness or contact with rubella or who were suspected of having congenital rubella.

In addition, sera from 44 patients with recent infection with other microbial agents and 12 sera containing rheumatoid factor detectable by latex agglutination, together with rubella specific IgG, were tested. None of these sera was positive by MACRIA. Nine sera from patients with infectious mononucleosis were evaluated, three having been shown to be reactive by MACRIA.

MACRIA
MACRIA was performed as previously described. Sera were measured in arbitrary units of rubella specific IgM. Concentrations of <1·0 arbitrary unit were considered to be negative, those 1·0–3·3 arbitrary units to be equivocal, those >3·3–9·9 arbitrary units to be low positive, and those >10 arbitrary units to be high positive.

PUBLIC HEALTH LABORATORY SERVICE MACELISA
The control sera in this assay were the same as those used for MACRIA—that is, negative, 1·0, 3·3, 10 and 40 arbitrary units. These sera, together with the test sera, were prediluted 1/100 by adding 10 µl of serum to 990 µl of phosphate buffered saline containing 0·05% Tween 20 (PBST). The assay was performed in polystyrene flat bottomed microtitre plates, the wells of which had been coated with anti-

Table 1 Detection of rubella specific IgM by Public Health Laboratory Service MACELISA in sera positive (>3·3 arbitrary units) by MACRIA

<table>
<thead>
<tr>
<th>MACRIA</th>
<th>No tested</th>
<th>Public Health Laboratory Service MACELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Positive, arbitrary units (&gt;3·3)</td>
</tr>
<tr>
<td></td>
<td>(1)</td>
<td>(2)</td>
</tr>
<tr>
<td><strong>Primary postnatal rubella:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High positive (&gt;10 arbitrary units)</td>
<td>134</td>
<td>134</td>
</tr>
<tr>
<td>Low positive (&gt;3·3–9·9 arbitrary units)</td>
<td>23</td>
<td>21</td>
</tr>
<tr>
<td><strong>Congenital rubella:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High positive (&gt;10 arbitrary units)</td>
<td>19</td>
<td>19</td>
</tr>
<tr>
<td>Low positive (&gt;3·3–9·9 arbitrary units)</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td><strong>After rubella immunisation:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High positive (&gt;10 arbitrary units)</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Low positive (&gt;3·3–9·9 arbitrary units)</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>188</td>
<td>184</td>
</tr>
</tbody>
</table>

(1) = King's College Hospital.
(2) = Division of Microbiological Reagents and Quality Control.

Table 2 Detection of rubella specific IgM by Public Health Laboratory Service MACELISA in sera equivocal (1·0–3·3 arbitrary units) by MACRIA

<table>
<thead>
<tr>
<th>MACRIA</th>
<th>No tested</th>
<th>Public Health Laboratory Service MACELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Positive, arbitrary units (&gt;3·3)</td>
</tr>
<tr>
<td></td>
<td>(1)</td>
<td>(2)</td>
</tr>
<tr>
<td>Congenital rubella*</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>After rubella immunisation</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Group equivocal by MACRIA</td>
<td>27</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>30</td>
<td>1</td>
</tr>
</tbody>
</table>

*Confirmed by persistent rubella specific IgG.
(1) = King's College Hospital.
(2) = Division of Microbiological Reagents and Quality Control.
human IgM. The prepared plates were stored wet with 50 \mu l of storage buffer in each well (estimated shelf life six months). After tipping out the storage buffer and tapping dry 100 \mu l of the prediluted test and control sera were carefully added to the bottom of the duplicate wells. The plate was covered with transparent adhesive tape and incubated at 37\degree C for two hours. The plate was then washed four times with PBST and blotted dry by inversion on to porous paper. Rubella haemagglutinin was diluted 1/20 in PBST and 100 \mu l added to each well and the plate was sealed as before and incubated overnight at room temperature. The plate was washed four times as above. Peroxidase conjugated monoclonal anti-rubella antibody was diluted as recommended in PBST containing 1% normal human serum not containing rubella antibody and 1% normal swine serum, and 100 \mu l was added to each well. The plate was sealed and incubated for two hours at 37\degree C. Towards the end of this incubation period the substrate ABTS (2,2' azino di (3 ethyl benzthiazoline sulphonic acid)) was prepared. Citrate phosphate buffer (5 ml), pH 4-0 at room temperature, was diluted with 5 ml of distilled water. To this diluted buffer 8 mg ABTS and 100 \mu l hydrogen peroxide (20 vols) were added. The substrate was left at room temperature in the dark until use.

After washing and blotting the plate 100 \mu l of substrate was added to each well and the plate incubated at room temperature in the dark for 30 minutes. The reaction was then stopped with 100 \mu l of a sodium fluoride solution, and each well was read at 405 nm with a spectrophotometer. A mean absorbance value was obtained for each serum and compared with the results obtained from the control sera. As for MACRIA, a concentration <1 arbitrary unit was considered to be negative, 1-0-3-3 arbitrary units to be equivocal, and >3-3 arbitrary units to be positive.

### Results

Tables 1–3 show the results of testing 403 sera from patients with recent primary postnatal rubella, congenital rubella, remote rubella, and postrubella immunisation, and include sera that had given an equivocal result by MACRIA. Of the 188 sera positive by MACRIA (Table 1), 187 were positive by the Public Health Laboratory Service MACELISA at test centre 2 and 184 at test centre 1. Two of the low positive sera collected from patients with recent primary postrubella rubella gave an equivocal result at test centre 1. One low positive serum from a patient with congenital rubella gave a negative result at test centres 1 and 2. A further serum from this group gave an equivocal result at test centre 1.

The two sera from patients with congenital rubella, confirmed by persistent rubella specific IgG, and which had given an equivocal result by MACRIA (Table 2), were negative by the MACELISA. The one serum collected after immunisation that had given an equivocal result by MACRIA was also equivocal by the MACELISA at test centre 2 but was negative at test centre 1. Of the group of 27 sera selected because they had given an equivocal result by MACRIA, four were positive, 18 were equivocal, and five were negative when tested at test centre 2. Of the four positive sera, one was also positive at test centre 1, but the three others gave an equivocal result. The five sera negative at test centre 2 were also negative at test centre 1. The laboratory at test centre 1 obtained a negative result with a further seven of the sera equivocal by MACRIA that had given an equivocal result by MACELISA at test centre 2.

All 185 sera which were rubella specific IgM negative by MACRIA gave a negative result by the MACELISA when tested at test centre 1. Nine, however, gave an equivocal result at test centre 2.
Antibody capture enzyme linked immunosorbent assay for detecting rubella specific IgM

Table 3.

Three of the 44 sera from patients with recent infection with other microbial agents gave an equivocal result by MACELISA at test centre 2. One of these was also equivocal by this method at test centre 1, but the two others were negative. Of the nine infectious mononucleosis sera, one (positive by MACRIA) was positive by the MACELISA at both laboratories, five were equivocal at test centre 2, and two were equivocal at test centre 1. The sera containing rheumatoid factor and rubella specific IgG were negative by the MACELISA.

Discussion

MACRIA has an appropriate sensitivity and specificity for detecting rubella specific IgM in primary postnatal8 and congenital rubella12 and is thus a useful standard for evaluating other techniques. Such an evaluation, however, and in particular that of the MACELISA reported here, must take into account the apparent, although uncommon, fallibility of MACRIA. MACRIA results in the range of 1-0-3.3 arbitrary units should be regarded as equivocal as they may be due to low concentrations of rubella specific IgM or, more commonly, they may be non-specific. Concentrations of >3.3 arbitrary units are usually considered to be positive, but these concentrations may occasionally occur when there is no supporting evidence of recent primary postnatal or congenital rubella. Indeed, MACRIA may detect rubella specific IgM in reinfections3 that are currently considered to be of minimal risk to the fetus.14 Thus an interpretation of the results must take into account all laboratory and clinical information.

The MACELISA was performed comfortably within two working days and was an easy and reproducible assay. As the coated microtitre plate is a relatively low cost component of the assay it should be possible to provide extra coated plates. This would enable sera to be tested when required rather than waiting for the accumulation of sufficient sera to use a plate fully. A further development may be the use of strips of coated wells.11

The MACELISA exhibited a similar sensitivity to MACRIA when detecting rubella specific IgM in primary postnatal rubella and after rubella immunisation. For sera positive by MACRIA from these groups discrepant results were observed with only two low positive sera that gave an equivocal result at test centre 1. The mean absorbance values obtained for these two sera at both laboratories were close to that of the 3-3 arbitrary units cut off serum.

Of further interest was the observation that one of the sera high positive by MACRIA gave a value of >10 arbitrary units by the MACELISA, although it had previously given low reactivity in Rubenza M,11 an essentially identical assay. This underlines the currently inexplicable variations in degree of reactivity that may occasionally occur when assaying a single serum for rubella specific IgM by several techniques. Although the Public Health Laboratory Service method detected rubella specific IgM in all sera high positive by MACRIA from patients with congenital rubella, only two of the four sera low positive by MACRIA gave a positive result in both laboratories. A third serum from this group gave a positive MACELISA result at one laboratory and an equivocal result at the other. Again, absorbance values obtained for this serum were close to that of the 3-3 arbitrary units positive control. The remaining serum gave a negative result by the Public Health Laboratory Service method and was from an asymptomatic neonate aged 12 days whose mother had had primary rubella at 27 weeks' gestation. MACRIA values of 5-0, 4-5, and 4-3 arbitrary units had been obtained and the serum was positive by Rubenza M. When it was tested by MACRIA in Manchester by Dr JE Cradock-Watson, however, rubella specific IgM was not detected. Thus this case of congenital rubella must now be considered unconfirmed but again shows the inexplicable differences in results that may be obtained between similar assays. The two sera equivocal by MACRIA from patients with congenital rubella were negative by MACELISA. This may reflect the reported lower sensitivity of the enzyme linked antibody capture assay compared with MACRIA for detecting rubella specific IgM in congenital rubella.12

On testing the 27 sera that had given equivocal results by MACRIA, four were positive by MACELISA. Three of these gave a positive result only at test centre 2. Two were acute sera with a low haemagglutination inhibition titre from patients with primary postnatal rubella, and the assay was, presumably, detecting low levels of rubella specific IgM. There was no supporting evidence of recent rubella either for the third serum positive only at test centre 2 or for the serum found to be positive in both laboratories. The absorbance values for these sera, however, were close to that obtained with the 3-3 arbitrary units positive control. Evidently, particular care should be given when assessing sera that yield absorbance values close to that of the 3-3 arbitrary units positive control.

Confirmation of the specificity of the MACELISA was shown by the absence of false positive results with the 182 sera from cases of remote rubella, even in the presence of a raised haemagglutination inhibition titre (>800 IU), and the 44 sera from cases of recent infection with other microbial agents. The
resistance of the assay to interference by rheumatoid factor was confirmed by the negative results with 12 sera containing rheumatoid factor and rubella specific IgG.

The results obtained by evaluating nine sera from patients with infectious mononucleosis show the problems that may be associated with such sera,16 one serum gave a positive result by the Public Health Laboratory Service method. This serum was reactive by MACRIA (20 arbitrary units) but when tested by Rubenz M gave an equivocal result.16 Two sera, which had been shown to be highly reactive (>3.3 arbitrary units) by MACRIA, however, were negative by the Public Health Laboratory Service method, illustrating once again that variation may be seen between similar assays.

In conclusion, the MACEILISA has a sensitivity equivalent to that of MACRIA except in occasional cases of congenital rubella with very low concentrations of rubella specific IgM. As with MACRIA, one or two sera gave a false positive result with the MACEILISA. These problems underline the necessity, as with all assays that detect rubella specific IgM, of interpreting the results in relation to all available clinical and serological data. It is also important that the degree of reactivity is correlated with the full findings to attempt to discriminate primary rubella from reinfection, and because false positive results are usually of low reactivity. The good agreement obtained between the two centres indicates that the assay is appropriately robust and can be recommended for routine diagnostic use.

Dr Hodgson was seconded from Dulwich Public Health Laboratory, Dulwich Hospital, London, throughout the period of this study.

References


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Public Health Laboratory Service
IgM antibody capture enzyme linked immunosorbent assay for detecting rubella specific IgM.
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