J. Hyg., Camb. (1981), **87**, 249
Printed in Great Britain

Comparison of radioimmunoassay and the gel filtration technique for routine diagnosis of rubella during pregnancy

By H. O. KANGRO,* C. JACKSON and R. B. HEATH
Department of Virology, The London Hospital, London, E1

(*Received* 7 *April* 1981)

SUMMARY

Radioimmunoassay (RIA) for rubella-specific IgM antibodies was compared with haemagglutination-inhibition (HI) in conjunction with gel filtration for the diagnosis of rubella infection in pregnant women during a 1-year period. In total 476 women were investigated of whom 221 were tested for rubella IgM. Both techniques gave positive results with 64 sera, and RIA alone with one additional serum.

Difficulties associated with the removal of non-specific HI activity were encountered with four sera all of which were negative by RIA.

RIA was found to have practical advantages over the gel filtration method but is at present technically more difficult to perform.

INTRODUCTION

Testing for specific IgM class antibodies plays an important and well-defined role in the routine investigation of possible rubella infection during pregnancy. Several methods have been described for the detection of these antibodies but the most commonly used techniques involve serum fractionation, either by sucrose density gradient centrifugation (Best, Banatvala & Watson, 1969) or gel filtration (Pattison & Mace, 1975), followed in both instances by the haemagglutination-inhibition (HI) test. Although these procedures have over the years proved reliable and generally satisfactory for routine diagnostic use, the need for serum fractionation and difficulties associated with the removal of non-specific HI activity (Pattison *et al.* 1978; Haukenes & Blom, 1975) are obvious drawbacks.

In recent years solid-phase radioimmunoassay (RIA) has been developed for the detection of rubella-specific IgM, which does not require prior serum fractionation or treatment for removal of inhibitors (Kangro, Pattison & Heath, 1978; Meurman, Viljanen & Granfors, 1977). So far, however, the potential usefulness of RIA as a diagnostic test for acquired rubella has not been assessed under routine diagnostic conditions.

During the rubella epidemic of 1978/79 in England and Wales we had the opportunity to compare RIA with the gel filtration-HI technique in routine diagnostic use and the findings are reported here.

* Present address: Department of Virology, St Bartholomew's Hospital, London, EC1.

MATERIALS AND METHODS

Serum specimens. These were submitted between May 1978 and May 1979 to the Virology Department from the antenatal clinics of the London Hospital Group for investigation of possible rubella infection during pregnancy.

Routine serological tests. The serological investigation of patients presenting with a rubella-like illness or contact followed the procedure that has been described in detail by Pattison & Dane (1975).

Rubella HI tests on unfractionated sera were performed using overnight incubation according to the method of Pattison & Mace (1973) except that single unit volumes of 0·5 % day-old chick erythrocytes (Tissue Culture Services Ltd) were used.

Testing for rubella-specific IgM antibodies by HI following gel filtration with Sephadex G-200 (Pharmacia Fine Chemicals Ltd) was performed according to Pattison & Mace (1975). The usual precautions were taken to avoid false positive results by this method (Pattison, Mace & Dane, 1976). The IgM peak fractions of some sera were tested after treatment with 2-mercaptoethanol (Caul, Smyth & Clarke, 1974) to destroy IgM antibody activity.

RIA procedure. The RIA procedure for rubella-specific IgM antibodies has previously been described (Kangro *et al.* 1978). Serum specimens were initially screened untreated at 1 : 400 and 1 : 1600 dilutions. Those sera which gave a positive result (Kangro *et al.* 1978) were then treated with latex-IgG beads (Cradock-Watson *et al.* 1979), to remove any possible reactivity due to rheumatoid factor (RF), and titrated in serial four-fold dilutions. Antibody titres were determined graphically by interpolation (Kangro *et al.* 1978). The fractions obtained by gel filtration for HI determination were also tested for specific IgM antibodies by RIA as previously described (Kangro *et al.* 1978).

RESULTS

During the 12-month study period 476 pregnant women were investigated serologically for possible rubella infection. The findings on initial HI testing necessitated subsequent serum fractionation and tests for IgM class antibodies in 194 (40·8 %) cases to confirm or exclude recent rubella infection. In addition to these cases IgM antibody tests were requested in 27 cases who had shown seroconversion or a significantly rising HI antibody titre in order to confirm a recent primary infection. The details of these 221 cases which were tested for rubella-specific IgM are summarized in Table 1.

Patients with a rubella contact

Of the 194 cases requiring serum fractionation 127 (65·5 %) were investigated because of contact with rubella or a rubella-like illness. Their serum specimens had high HI antibody titres and were taken at least 10 days after contact in 113 cases, or in 14 cases after a rubella-like illness in a family member, most commonly (13 cases) in a young child from a previous pregnancy. Rubella-specific IgM was detected by both RIA and the gel filtration technique in only 11 (8·7 %) of these 127 sera.

Table 1. *Summary of the results of laboratory tests on 221 sera that were fractionated and tested for rubella-specific IgM antibodies*

Group	Time of specimen after contact or onset (days)	HI antibody titre	Number of cases	Specific IgM detectable	
				HI after fractionation	RIA on whole serum
Contact with rubella-like illness					
Casual contact	10–90	256–4096	113	9 (+4)*	9
Family contact	2–28	256–4096	14	2	2
Rubella-like illness					
Rash present	1–60	128–4096	47	28	28
Rash absent	7–22	128–4096	11	4	4
Others	—	128–4096	9	2	2
Seroconversion or rising titre	—	256–⩾ 4096†	27	19 (+1)	19 (+1)
			221	64	64

* Figures in brackets are results obtained with six sera that gave discordant results.

† Titres of second sera from these patients.

Patients with a rubella-like illness

Fifty-eight (29·9%) cases presented 1–60 days after onset of symptoms suggestive of rubella infection. A rubelliform rash was the predominant feature in 47 cases of which 28 (59·6%) were confirmed as rubella by the detection of specific IgM. Of the 19 IgM negative cases 3 were subsequently diagnosed as measles and one as parotitis.

The 11 cases presenting without a rash comprised five with joint pains, three with cervical lymphadenopathy, two with upper respiratory tract infections and one with purpura. Four of these cases (two with joint pains and two with lymphadenopathy) had detectable rubella-specific IgM.

Patients tested for reasons other than contact or illness

Nine women with high rubella HI titres were tested for specific IgM for various reasons. Five women were investigated post-partum because of congenital abnormalities in their babies. Rubella-specific IgM was detected in one of these women.

In two cases laboratory investigation was sought after inadvertent rubella vaccination during pregnancy and specific IgM was detectable in one case. Of the remaining two cases one was investigated because of threatened abortion and one because of an 8-fold rise in the HI titre (from 32 to 256) between booking and third trimester but no specific IgM was detected in either of these cases.

Sera from patients who seroconverted or showed diagnostic rising HI titres

Of the 27 women who seroconverted or showed diagnostic rises in the HI titres 10 presented within four days of a rubella-like illness and four because of contact with a rubella-like illness 2–3 weeks previously. The second sera from these 14 women gave HI titres of 256–⩾ 4096 and all contained rubella-specific IgM. Unfortunately the acute phase sera were not available for testing by RIA. The

Table 2. *Discordant results between the RIA and HI tests obtained with six sera*

Serum	Serum fractions		Whole serum
	HI	RIA	RIA
1	+	−	−
2	+	−	−
3	+	−	−
4	+	−	−
5	+	−	−
6	−	+	+

+ Denotes positive result.
− Denotes negative result.

remaining 13 women were susceptible to rubella by HI at the time of booking but gave titres of 256–4096 post-partum. However, on testing a post-partum specimen, rubella IgM antibodies were detectable by both test methods in only five of these 13 cases.

Sera giving discordant results

Six sera gave discordant results with the two IgM tests (Table 1). These are shown in more detail in Table 2. Sera 1–4 were obtained from contacts whereas sera 5 and 6 were post-partum specimens from women who had seroconverted during pregnancy. Sera 1–5 gave peak HI titres of 1–4 in the IgM containing fractions but no rubella-specific IgM was detectable by RIA either with whole serum or IgM fractions. The HI activity was removed from sera 1, 2 and 4 when $MnCl_2$/heparin treatment and serum fractionation were repeated whereas in sera 3 and 5 it persisted. However, in these two sera the HI activity was resistant to treatment with 2-mercaptoethanol and was thus not due to IgM antibody. In serum 5 (HI = 4096) the inhibitory activity was at the foot of the ascending limb of the IgG peak, which extended into the IgM peak, and was probably therefore due to IgG or IgA class antibody.

In serum 6, rubella-specific IgM was detected by RIA but not by HI. The RIA titre with whole serum was low (1000), and since this serum was from a patient who seroconverted during pregnancy it is likely that this is a true result and reflects the greater sensitivity of RIA.

IgM antibody titres

Rubella-specific IgM was detected in altogether 64 (29%) of the 221 sera tested by both RIA and the gel filtration method. The RIA titres ranged from 800 to $\geqslant$ 51200 and showed very good correlation with the corresponding peak HI titres of the IgM fractions (Fig. 1). A reasonably accurate ($\pm$3 days) history of onset of symptoms could be obtained in 51 cases which showed that high titres ($>$ 10000) were only found up to 6 weeks after onset. Forty-four sera were taken within 4 weeks after onset of symptoms and gave a median titre of 22000. Only 5 (11·4%) of these sera gave titres of $<$ 6400 and none $<$ 3200. IgM titres of $<$ 1600 were only obtained with sera taken more than 6 weeks after onset of symptoms.

Specificity and reliability of the RIA-IgM test. There was complete agreement between the RIA-IgM tests on whole serum and the corresponding IgM fractions, 65 sera giving positive results while 156 sera were negative. Furthermore, RIA on

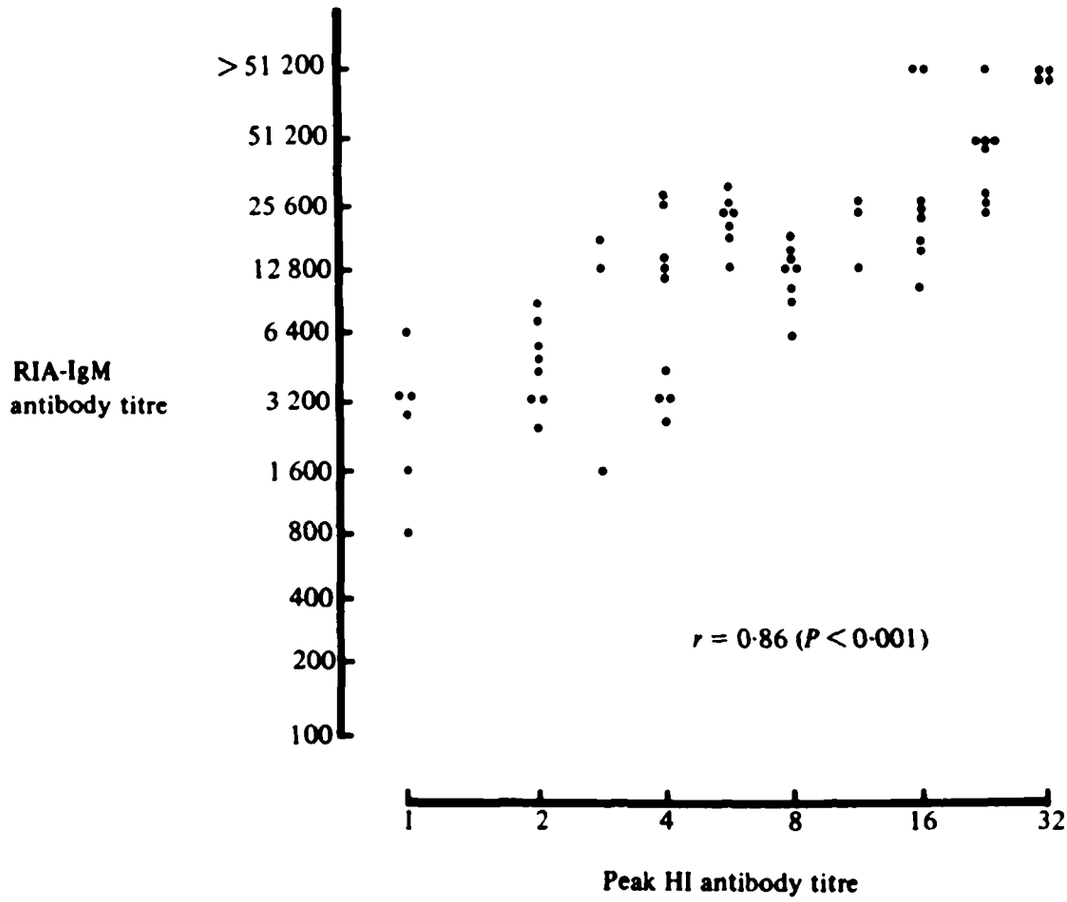

Fig. 1. Correlation between RIA-IgM antibody titres in whole serum and the corresponding peak HI titres in the IgM containing fractions.

the serum fractions confirmed the presence of IgM class antibodies detected by HI, although RIA titres were 10–200 times higher (mean: 77 times) than HI titres.

Latex-IgG treatment showed evidence of RF activity in only one serum which was positive by RIA on screening. This serum gave an RIA-IgM titre of 12800 and exceptionally high counts at the lower dilutions with the untreated serum. Absorption with latex beads reduced the counts by approximately 2-fold with the 1/100 and 1/400 serum dilutions but had no effect on either the counts with higher serum dilutions or the IgM titre.

The reliability of the RIA-IgM test was further assessed in 78 of the 255 cases that were not fractionated and tested for rubella-specific IgM. The sera from these 78 cases were obtained more than 10 days after contact with a rubella-like illness and gave HI titres of 16–128. Second specimens were therefore requested from these patients but none showed a diagnostic rise in the HI titre. Both serum specimens from these 78 cases were tested by RIA and all were negative for rubella-specific IgM.

DISCUSSION

A confident laboratory diagnosis of rubella during pregnancy often depends on the detection of specific IgM antibodies (Pattison & Dane, 1975). During the 1-year period when RIA and the gel filtration technique were compared 40·8% of the cases submitted for investigation of possible rubella infection required IgM antibody tests. There was almost complete agreement between the two techniques. Thus,

rubella-specific IgM was detected by both methods in 64 (29%) of the 221 sera tested. The higher degree of sensitivity of RIA was of apparent diagnostic value in only one case but in this patient the infection had also been diagnosed by seroconversion. A potentially more likely source of a false result in the gel filtration technique is associated with the removal of non-specific inhibitors in the HI test. Five sera in this study were positive by HI but negative by RIA. With four sera the results obtained on repeated testing indicated that the HI activity observed in the IgM containing fractions was due to unsuccessful removal of non-specific inhibitors. Whether the results with any of these sera would have been wrongly interpreted as indicative of a recent rubella infection is difficult to assess but at least two of these sera would normally have been retested because they gave atypical HI patterns.

These results clearly demonstrate that in diagnostic practice the number of cases where the RIA-IgM test would prove more reliable than the gel filtration-HI technique is very small and RIA is therefore unlikely to replace the techniques currently used in most diagnostic laboratories. If however, future routine diagnosis of rubella were to be centralized the practical advantages of RIA could make it an attractive alternative, especially as a rapid screening test to separate large numbers of IgM antibody positive and negative sera. In this study 149 (76·8%) of 194 sera that required fractionation and testing for specific IgM antibodies gave negative results. Furthermore, the higher degree of sensitivity in RIA may allow detection of IgM antibodies earlier after infection than is possible by conventional serology. Thus, Meurman *et al.* (1977) detected specific IgM in seven of 20 acute phase sera, taken < 4 days after onset of rubella, by sucrose density gradient centrifugation and HI but in nine of the same 20 sera by RIA. Whether this could reliably be used to confirm or exclude rubella infection in patients who present with low HI titres, and therefore normally are requested to donate second specimens, could not be fully evaluated. Altogether 78 seropositive contact cases in this study in whom paired sera were tested showed no evidence of rubella infection and this could have been excluded by the absence of specific IgM in the first serum.

As previously reported (Kangro *et al.* 1978; Cradock-Watson *et al.* 1979) a problem encountered with some adult sera is the significant binding of radiolabel with the control antigen at low serum dilutions which depresses virus-specific binding. The distinction between antibody positive and negative sera is thus reduced but we have no evidence that the virus-specific binding is depressed to such an extent that low levels of IgM antibodies are masked.

RF is reactive in RIA and sera have to be absorbed before positive results can be relied upon. Although 2% of women of childbearing age have RF (Lawrence, 1965) only one serum showed evidence of RF activity in this study and this also contained rubella-specific IgM. RF does therefore not present a major problem in the RIA-IgM test when used for the diagnosis of acquired rubella.

REFERENCES

BEST, J. M., BANATVALA, J. E. & WATSON, D. (1969). Serum IgM and IgG responses in postnatally acquired rubella. *Lancet* ii, 65.

CAUL, E. O., SMYTH, G. W. & CLARKE, S. K. R. (1974). A simplified method for the detection

of rubella-specific IgM employing sucrose density gradient fractionation and 2-mercaptoethanol. *Journal of Hygiene* **73**, 329.

CRADOCK-WATSON, J. E., RIDEHALGH, M. K. S., PATTISON, J. R., ANDERSON, M. J. & KANGRO, H. O. (1979). Comparison of immunofluorescence and radioimmunoassay for detecting IgM antibody in infants with the congenital rubella syndrome. *Journal of Hygiene* **83**, 413.

HAUKENES, G. & BLOM, H. (1975). False positive rubella virus haemagglutination inhibition reactions: occurrence and disclosure. *Medical Microbiology and Immunology* **161**, 99.

KANGRO, H. O., PATTISON, J. R. & HEATH, R. B. (1978). The detection of rubella-specific IgM antibodies by radioimmunoassay. *British Journal of Experimental Pathology* **59**, 577.

LAWRENCE, J. S. (1965). In *Progress in Clinical Rheumatology*. (ed. Dixon, A. J.), p. 1. London: J. & A. Churchill Ltd.

MEURMAN, O. H., VILJANEN, M. K. & GRANFORS, K. (1977). Solid-phase radioimmunoassay of rubella virus immunoglobulin M antibodies. Comparison with sucrose density gradient centrifugation test. *Journal of Clinical Microbiology* **5**, 257.

PATTISON, J. R. & DANE, D. S. (1975). The routine serological investigation of cases and contacts of rubella. *Journal of Hygiene* **75**, 91.

PATTISON, J. R., JACKSON, C. M., HISCOCK, J. A., CRADOCK-WATSON, J. E. & RIDEHALGH, M. K. S. (1978). Comparison of methods for detecting specific IgM antibody in infants with congenital rubella. *Journal of Medical Microbiology* **11**, 411.

PATTISON, J. R. & MACE, J. E. (1973). Rubella screening tests. *Journal of Clinical Pathology* **26**, 161.

PATTISON, J. R. & MACE, J. E. (1975). The detection of specific IgM antibodies following infection with rubella virus. *Journal of Clinical Pathology* **28**, 377.

PATTISON, J. R., MACE, J. E. & DANE, D. S. (1976). The detection and avoidance of false-positive reactions in tests for rubella-specific IgM. *Journal of Medical Microbiology* **9**, 355.