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A simplified method for the detection of rubella-specific IgM employing sucrose density fractionation and 2-mercaptoethanol

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SUMMARY

A simplified method for the detection of rubella-specific IgM in sera was developed involving HAI tests on only one fraction from a sucrose density gradient with and without treatment with 2-mercaptoethanol (2 ME). Slight trailing of IgG into the IgM fraction was shown to occur in patients with high titres of both IgG and IgM, but this could be detected by the 2ME treatment and did not affect the results.

By this test, rubella-specific IgM was found in all of 54 patients with a rash and a rise of rubella HAI antibodies. When the antigen and serum were incubated for 1 hr. before adding the RBC, specific IgM was found in all 51 sera taken between 5 and 40 days after onset, and in about half the sera taken between 42 and 77 days, but in none of 16 people known to have possessed rubella antibodies at least a year previously. When antigen and serum were incubated for 18 hr. before adding the RBC, specific IgM was found in all of 21 sera taken between 2 and 49 days after onset, and in 6 of 11 sera taken between 50 and 117 days, but not in 9 people known to have possessed rubella antibodies at least a year before. The method has been found to be very useful in detecting recent rubella among patients in early pregnancy.

INTRODUCTION

A common problem in virology is the diagnosis of patients in early pregnancy who are not seen until more than a week after a rash or more than three weeks after contact with a case of rubella. Conventional laboratory tests may not help to decide whether the patient has had rubella during her pregnancy. The presence of rubella-specific IgM immunoglobulin seems to indicate recent infection (Vesikari & Vaheri, 1968; Best, Banatvala & Watson, 1969; Desmyter, South & Rawls, 1971; Ogra *et al.* 1971; Cradock-Watson, Bourne & Vandervelde, 1972; Haire & Hadden, 1972; Field & Murphy, 1972; Forghani, Schmidt & Lennette, 1973). This has been detected by 2-mercaptoethanol (2ME) treatment of whole serum (Banatvala *et al.* 1967), immunofluorescence (Baublis & Brown, 1968; Cohen, Ducharme, Carpenter & Deibel, 1968), separation of immunoglobulins in sucrose density gradients followed by rubella haemagglutination inhibition (HAI) (Vesikari & Vaheri, 1968) or radioimmunodiffusion (Ogra *et al.* 1971), separation in a Sephadex column (Gupta, Peterson, Stout & Murphy, 1971) or agarose (Bürgin-Wolff, Hernandez & Just, 1971) followed by HAI.

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Each of these techniques has disadvantages. With sucrose gradient separation there may be 'trailing' of IgG into the IgM fraction, and the technique is laborious. We tried to overcome the first difficulty by doing HAI tests with and without treatment of the individual fractions with 2ME, which disrupts IgM; we tried to reduce the labour by collecting and testing only one fraction after a careful calibration of the system. This simplified test was done on sera from 54 proved cases of acquired rubella and 16 normal adults who were known to have possessed rubella antibodies at least a year before. Most workers have used normal adults as control subjects, but it is possible that these people may be having subclinical rubella. After our method had been developed, Field & Murphy (1972) described a similar technique.

MATERIALS AND METHODS

Sera

These were collected from:

(1) Fifty-four patients with clinical rubella confirmed by a four-fold or greater rise of titre of rubella HAI antibodies. Six were children aged 7–15 years and 48 were adults of whom 19 were pregnant. Two sera were collected from each of 20 patients and three from each of 34. Sera from three of these patients were used for the calibration tests (see below). The date of onset of the rash was recorded as precisely as possible, but it was realized that in a few patients this information may have been inaccurate.

(2) Six patients whose serum contained rheumatoid factor as well as rubella HAI antibody.

(3) Sixteen laboratory staff who were known to have possessed rubella HAI antibodies at least 1 year previously. One of these sera was used in the calibration tests.

All sera were stored at -20° C. and examined within 18 months of collection without inactivation.

Preliminary treatment of sera

Before gradient separation, 0·25 ml. of serum was mixed with 0·25 ml. of dextrose–gelatin–veronal buffer (DGV) and 0·05 ml. of 50 % suspension of day-old chick RBC, incubated for 1 hr. at 4° C. and centrifuged to remove the RBC. A standard serum containing rubella-specific IgM was included in every run.

Sucrose density centrifugation

Sucrose solutions of 12·5, 18·75, 25·0, 31·0 and 37·5 % (w/v) were made in DGV and kept at 4° C. Discontinuous gradients were made by layering 0·7 ml. amounts of each solution into 5 ml. MSE polycarbonate ultracentrifuge tubes. Immediately, 0·4 ml. of treated serum was placed on top. The tubes were then centrifuged in a 6×5 titanium swing-out rotor in an MSE superspeed 75 ultracentrifuge for 18 hr. at 4–5° C. at 100,000 g with the brake off. Fractions were collected at once with an MSE tube piercer. Twelve fractions each of 25 drops

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(0·3 ml.) were collected and used for the early calibrations. In later routine estimations only the second 50 drops (pooled fractions 3 and 4) were collected.

Rubella HAI tests

The fractions were divided into two equal volumes; to one was added 0·05 ml. of 0·5 M 2ME (made up fresh every three weeks and stored at 4° C.), and to the other 0·05 ml. of DGV. Both were incubated in a water bath at 37° C. for 1 hr. and the samples were then titrated for rubella HAI antibodies in a microtitre test with 4HA units of antigen, 0·16 % day-old chick RBC and DGV. The antigen and serum dilutions were allowed to react for 1 hr. (short method) or for 18 hr. (long method). The tests were read after the RBC had settled for 3 hr.

HAI tests on whole sera were done in the same way, except that non-specific inhibitors were first removed with kaolin, 2ME was not used, and short fixation was always used.

Detection of IgA, IgG and IgM immunoglobulins

The fractions were tested by immunodiffusion in an agarose gel against anti-IgA, -IgG or -IgM sera (Hyland). The plates were examined at 1, 2 and 3 days and the amount of precipitate roughly estimated by eye and graded as ±, +, ++ or +++.

RESULTS

Calibration of centrifugation technique

A detailed examination was made of sera from three patients with clinical rubella and a rise in HAI antibodies, and from one normal subject. After separation in sucrose density gradients, all 12 fractions were examined for rubella HAI antibodies by the short method with and without treatment of the fractions with 2ME, and each fraction was tested for total IgA, IgG and IgM by immunodiffusion. The immunodiffusion test was read after three days in order to detect very small amounts of immunoglobulins which might have trailed into neighbouring fractions.

In patient A (Fig. 1) one day after the onset of the rash there was a low titre of antibody in fractions 3 and 4, which was completely removed by 2ME. These two fractions contained maximum IgM in the immunodiffusion tests and it was thought that all the rubella antibody was IgM. There was a low titre of antibody in fractions 6, 7 and 8 which was unaffected by 2ME, and immunodiffusion tests showed that these fractions contained IgA and IgG, but no IgM. Fractions 9, 10 and 11 had higher HAI titres than the other fractions, which were unaffected by 2ME. These fractions were thought to contain lipoprotein non-specific inhibitor mixed with a little IgG.

In patient A 11 days after the onset of the rash (Fig. 2) the titres of fractions 3, 4 and 5 were reduced by 2ME, showing that all three contained IgM. However, in fractions 4 and 5 antibody was not removed completely, showing that some IgG had been brought down into these two fractions. A similar result was seen with another patient B 5 days after onset, and with patient C 11 days after onset (Fig. 3). Patient C had a higher titre of rubella antibodies in both the IgM and

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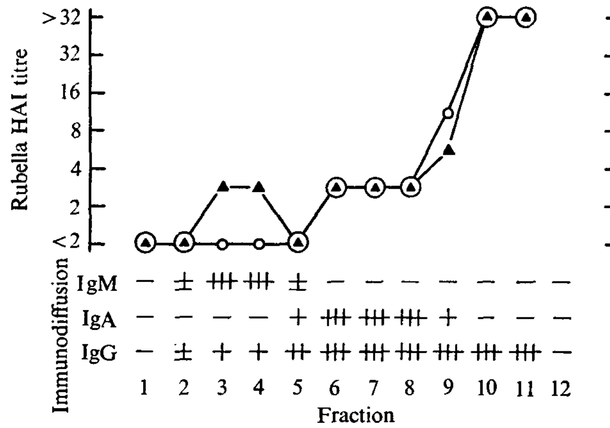

Fig. 1. Immunodiffusion against anti-IgM, IgA and IgG and rubella HAI on sucrose gradient fractions of serum from patient A taken 1 day after rash. ▲—▲ = without 2ME treatment; ○—○ = with 2ME treatment; – = no precipitate; ±, +, + +, + + + = amount of precipitate estimated by eye.

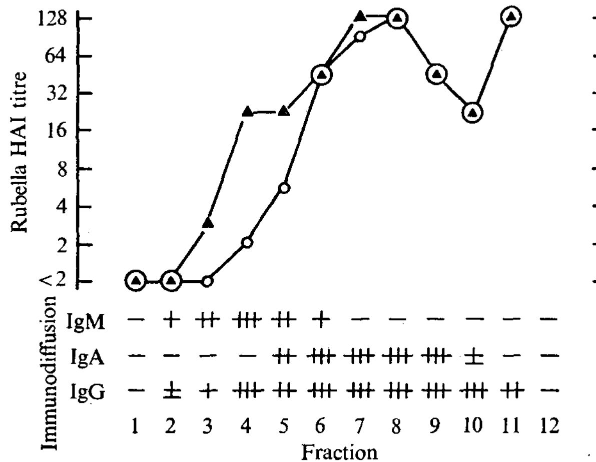

Fig. 2. Immunodiffusion against anti-IgM, IgA and IgG and rubella HAI of sucrose gradient fractions of serum taken from patient A 11 days after rash. ▲—▲ = without 2ME; ○—○ = with 2ME; – = no precipitate; ±, +, + +, + + + = amount of precipitate estimated by eye.

IgG fractions. However, when subject D (Fig. 4), who was known to have possessed rubella antibodies at least seven years before, was tested, all the rubella antibodies were in fractions 6 and upwards and all were unaffected by 2ME. The rubella antibodies were localized in the IgG fractions 6, 7 and 8 with no spread into either the lower IgM fractions or the higher non-specific inhibitor fractions. A similar result was found with serum from another normal subject.

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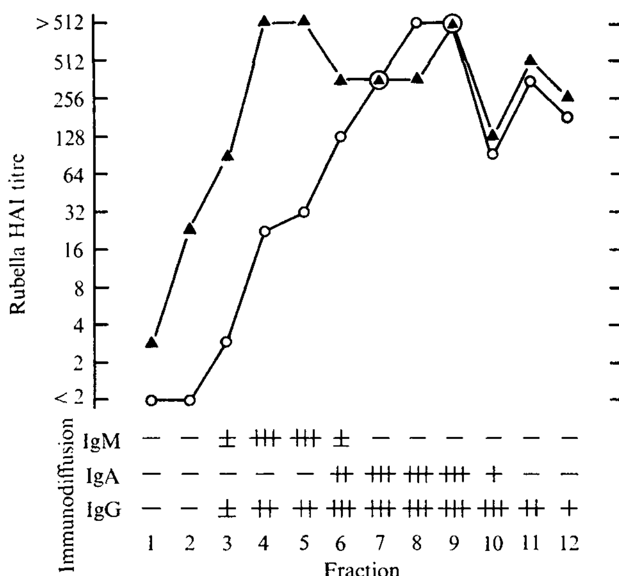

Fig. 3. Immunodiffusion against anti-IgM, IgA and IgG and rubella HAI of sucrose gradient fractions of serum from patient C taken 11 days after rash. ▲—▲ = without 2ME; ○—○ = with 2ME; − = no precipitate; ±, +, + +, + + + = amount of precipitate estimated by eye.

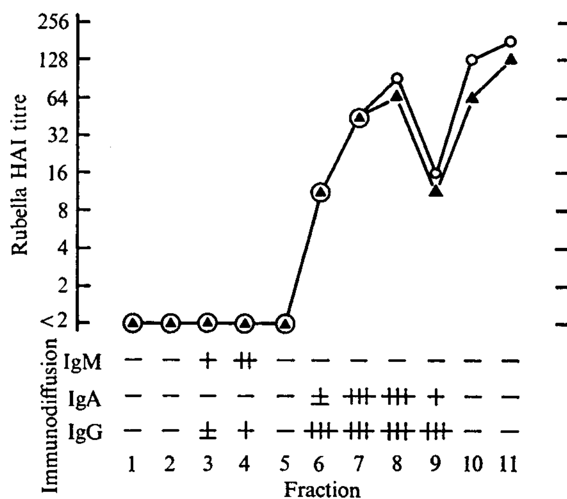

Fig. 4. Immunodiffusion against anti-IgM, IgA and IgG and rubella HAI of sucrose gradient fractions of serum from subject D. ▲—▲ = without 2ME; ○—○ = with 2ME; − = no precipitate; ±, +, + +, + + + = precipitate estimated by eye.

Identification of the residue

To characterize the residual inhibitor after 2ME treatment of fractions 3 and 4, these fractions pooled from a serum containing a high titre of IgM were treated with and without 2ME. Each lot was then divided into four parts and treated with an equal volume of phosphate-buffered saline, or of antiserum to either IgA,

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IgG or IgM. After incubation for 1 hr. at 37° C., these mixtures were tested for rubella HAI antibody. In the part without antiserum there was residual activity after treatment with 2ME, but after mixing with anti-IgG this disappeared. Anti-IgM reduced the titre before treatment with 2ME but not after. Anti-IgA did not affect the titres. From this it was concluded that the residue was IgG.

Simplified test for rubella-specific IgM

From these results it was decided to collect the second 50 drops from the sucrose gradient (fractions 3 and 4 pooled) and not other fractions. Rubella HAI tests were done with and without treatment with 2ME. Any inhibition which was reduced at least 3-fold by the 2ME was considered to represent rubella-specific IgM. Fractions were not routinely tested by immunodiffusion.

All sera were tested without preliminary inactivation, although it was shown on one occasion that inactivation for 30 min. at 56° C. did not affect the IgM titre.

Length of incubation

After most of the tests described below had been done by the short method, it was suggested that incubation of the antigen and serum overnight at 4° C. before adding the RBC might make the test more sensitive (Pattison & Mace, 1973). In a comparison of the long and short methods on 20 sera containing IgM, the long method resulted in 2- to 8-fold higher titres (mean: 4-fold).

Reproducibility

Repeated tests on sera containing IgM showed only a 4-fold variation in 13 tests and a 2-fold variation in 8 tests by the short method, and a 4-fold variation in 7 tests and a 3-fold variation in 14 tests by the long method.

Rubella-specific IgM titres in patients with rubella

Including the 3 patients on whom the calibration tests were done, rubella-specific IgM was measured in a total of 54 patients. All had had a rash and shown a 4-fold or greater rise in rubella HAI on whole serum. The IgM test was done on 1 serum from each of 15 patients, 2 sera from each of 25 patients and 3 sera from each of 14 patients. Sera taken early in the illness, where the HAI titre of whole serum was less than 24, were not usually tested for IgM. Because of technical difficulties, fraction 5 was tested instead of fractions 3 and 4 in six cases. Titres were similar to those found with other sera taken at the same time, and so have been included in the results. In 10 patients there was some doubt as to whether the date of onset of the rash was accurate; however, since the IgM titres were similar to those of other patients, these 10 have been included. Most of the sera were tested by the short method, but after it was shown that overnight incubation gave higher titres, sera taken between 42 and 74 days after onset which were negative by the short method were repeated by the long method. A few sera were tested only by the long method.

Rubella-specific IgM was found at some time in all 54 patients. The titres reached a peak 7 to 12 days after the rash and then gradually declined (Fig. 5).

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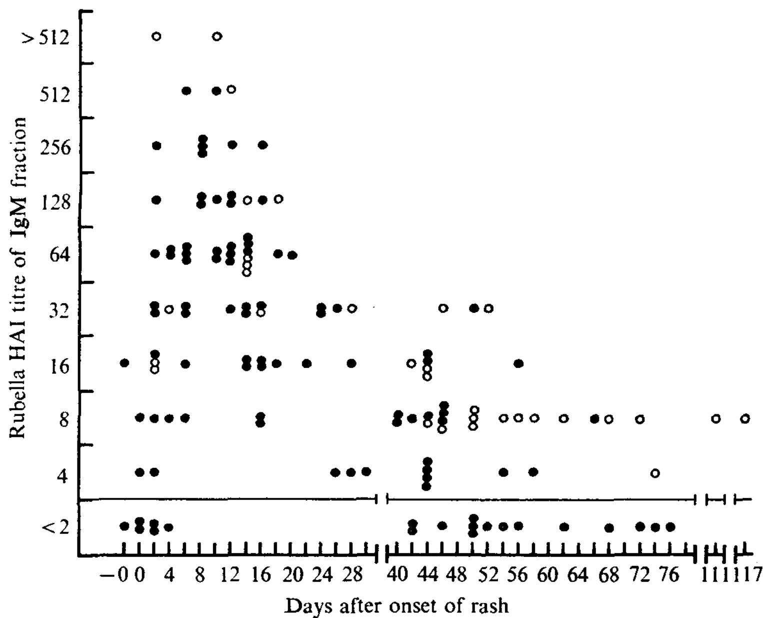

Fig. 5. Rubella HAI titres of IgM fractions of sera from 54 patients with proved rubella. ● = short method; ○ = long method. Figure includes 26 sera tested by both methods.

By the short method, specific IgM was found in all 51 sera taken between 5 and 40 days after the rash, and after 40 days it was found in about half. By the long method, specific IgM was found in 27 of 32 sera taken between 2 and 117 days after onset. In the remaining 5 sera taken between 50 and 72 days after onset, there was HAI in the IgM fraction, but there was less than a 3-fold fall in titre after treatment with 2ME. By the short method, the IgM fractions of 5 sera (taken 9, 11, 21, 50 and 65 days after onset) showed only a 3-fold fall in titre after treatment with 2ME. All the other sera showed a 4-fold or greater fall.

The residual antibody found after 2ME treatment was considered to be IgG brought down by the IgM. The amount of this residual antibody was greater the higher the total HAI titre of the whole serum, and the higher the rubella-specific IgM titre. It was never found where there was no IgM, or in early sera where there was little or no IgG.

Specific IgM titres among the pregnant patients were similar to those of the non-pregnant patients.

Normal adults

Sera were collected from 16 laboratory staff, 7 of whom were known to have possessed rubella antibody at least a year previously, 5 at least 2–3 years previously, and 4 at least 7 years previously. None had detectable rubella-specific IgM by the short method. Nine were tested by the long method and none had specific IgM, or any HAI in the IgM fraction.

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Rheumatoid factor

Sera from 6 patients known to contain rheumatoid factor all had fairly high levels of rubella HAI antibody. Rubella-specific IgM was detected in none by the short method.

DISCUSSION

Ultracentrifugation in a sucrose gradient followed by rubella HAI of the fractions was first described by Vesikari & Vaheri (1968) and later by Best *et al.* (1969). Both these groups of workers tested every fraction and Vesikari & Vaheri treated the sera with 2ME *before* fractionation to control the test. However, this is not a good control, since trailing will be eliminated in the control preparation but not in the test. Best *et al.* (1969) found specific IgM in all sera up to 20 days after onset, in a proportion of sera taken between 21 and 30 days and in none taken after 30 days. The test was simplified by Desmyter *et al.* (1971), who examined two fractions but detected no IgM after 36 days. Forghani *et al.* (1973) also tested only two fractions, and found specific IgM in all of 18 sera taken between 3 and 20 days after onset, and none of 10 subjects taken 1–30 years after rubella. A simplified test with only one fraction tested with and without 2ME similar to the one described here was developed by Field & Murphy (1972). In 6 patients with proved rubella, they found specific IgM in all of 8 sera taken 3–15 days after onset.

In the present study, the simplified method for detecting rubella-specific IgM worked well. It was not too time-consuming and the treatment of the fraction with 2ME gave little extra work. By the short method, IgM was found in all 49 sera taken between 5 and 30 days after onset, and in the 2 sera taken 40 days after onset. Thus there were no 'false negative' results, so that, when investigating patients with undiagnosed rashes on single late sera, failure to detect rubella-specific IgM between 5 and 30 days after onset means that it is unlikely that the illness is rubella.

There were also no 'false positive' results by the short method, in that 16 people known to have had rubella more than a year before showed no rubella-specific IgM. Six patients with rheumatoid factor, which is an IgM antiglobulin, and so can give false positive results by other techniques (Fraser, Shirodaria & Stanford, 1971; Cradock-Watson *et al.* 1972), showed no specific IgM by the present technique, although all had rubella antibodies. Desmyter (1972) likewise showed no false positive results with rheumatoid sera on sucrose gradients. It was found that in the concentration used in the present experiments, 2ME did not break down IgG and therefore give false positive results, for in all the IgG fractions in the calibration experiments, 2ME did not reduce the HAI titre, and in previous experiments with large numbers of whole sera from normal adults, treatment with 2ME did not affect the HAI titre. Cradock-Watson, Ridehalgh, Bourne & Vandervelde (1973) sometimes found IgA in the IgM fractions and suggested that early IgA might be partly in the polymeric form. This might give a false positive result, but would still indicate a recent infection. In the present work, IgA was not found in the IgM fraction.

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It is possible that if a patient's serum continued to have rubella-specific IgM for years after a primary infection, on rare occasions this might give a false positive result. Specific IgM occasionally persists in patients with chronic rubella infections such as thrombocytopaenia or carpal tunnel syndrome for up to 9 months (Haire & Hadden, 1970), or after vaccination (Gupta, Peterson & Murphy, 1972). Baublis & Brown (1968) and Desmyter *et al.* (1971) have described the occasional persistence of IgM for more than 6 months in mothers carrying a rubella-infected fetus.

The long technique described here is probably less helpful than the short, since, although more of the sera taken more than 40 days after onset showed specific IgM than by the short method, and although there were no positive results several years after rubella, it is possible that the technique detects low levels of IgM many months after the onset of a straightforward infection, so that the finding of a low level by this technique is difficult to interpret. An investigation of sera taken between 2 and 24 months after infection is in progress.

The long technique might be of value in detecting IgM following administration of rubella vaccines inadvertently during pregnancy, particularly when vaccinees present many weeks after vaccination.

In this laboratory, the short technique has been used on pregnant patients who had had a rash or been in contact with rubella, and has successfully distinguished 9 who had recently had rubella from 61 who almost certainly had not.

Recently, reinfection with rubella after either wild rubella or after rubella vaccination has been reported. During reinfection the fetus is probably less likely to be affected than during primary infection. It is unlikely that rubella-specific IgM would be found during reinfection, so the test would be helpful in these cases.

The short method sometimes failed to detect rubella-specific IgM less than 5 days after onset, but this does not matter in practice for sera can always be taken a few days later to detect a rise in HAI or complement-fixing antibody.

Since IgA is also found early after an infection, some workers have suggested that the detection of rubella-specific IgA would also help in these patients (Bürgin-Wolff *et al.* 1971; Cradock-Watson *et al.* 1972). By the present technique, IgA could not be separated from IgG. Field & Murphy (1972) also could not separate them by sucrose density centrifugation. Although Bürgin-Wolff *et al.* (1971) and Cradock-Watson *et al.* (1972) found that rubella-specific IgA disappeared at about the same time as IgM, Ogra *et al.* (1971) using selective absorption with anti-IgG and IgM found that specific IgA was still present at fairly high titre a year after infection. Tests for rubella-specific IgA might therefore not be helpful in the field.

Sucrose gradient centrifugation for the detection of rubella-specific IgM has been criticized by Newman, Horta-Barbosa & Sever (1969) and by Sever (1969) because of trailing of IgG in the IgM fractions, which is particularly marked when undiluted serum is used (Best & Banatvala, 1969; Desmyter *et al.* 1971; Field & Murphy, 1972). In the present work, some trailing occurred especially where there was a high titre of both rubella-specific IgM and IgG. IgG may also be mixed with the lower fractions by bad technique. Both trailing and mixing are controlled by treating the fractions with 2ME, which shows that the inhibition is due to

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IgM and not IgG. Forghani *et al.* (1973) suggested that 2ME is not satisfactory for confirming IgM in fractions because reaggregation may take place, but Field & Murphy and ourselves have found no difficulty in confirming IgM in the fractions with 2ME.

It is important to remove chick haemagglutinins before doing the test, but not non-specific inhibitors, since these remain at the top of the gradient and so do not interfere with the test. It is important not to use kaolin, since this may remove IgM. IgM is said to be inactivated at 56° C. and on storage at −20° C. In the present work, the titre of specific IgM was unaffected by 56° C. for 30 min. on one occasion, and sera which had been stored for up to 18 months at −20° C. often had high titres of specific IgM. The sucrose did not interfere with the HAI test, although the RBC took longer to settle, especially if they were old. This was overcome by reading all tests at 3 hr. instead of 2.

Rubella-specific IgM can be detected by immunofluorescence of cells infected with rubella examined with patient's serum and conjugated anti-human IgM serum (Baublis & Brown, 1968; Cohen *et al.* 1968; Haire & Hadden, 1970, 1972; Cradock-Watson *et al.* 1972, 1973). The best results were obtained by Haire & Hadden (1972), who found specific IgM in all sera up to 30 days after onset, in a proportion between 44 and 50 days and in none after 52 days. Sera containing rheumatoid factor give false positive results by this technique (Fraser *et al.* 1971) and another difficulty is the blocking of antigen by IgG antibody (Cradock-Watson *et al.* 1972). Specific fluorescence can be obtained only when the virus has been grown under special conditions (Haire, Adair & Fraser, 1972).

The separation of IgM on a Sephadex column and testing it for rubella HAI antibody with and without treatment of the fraction with 2ME was developed by Gupta *et al.* (1971, 1972) and used by Pead (1974). This technique requires less expensive equipment than sucrose density separation, but non-specific inhibitor may be a problem, and the fractions must be concentrated, and only one specimen can be processed each night.

Separation of IgM by filtration through agarose with rubella HAI of the fractions was described by Bürgin-Wolff *et al.* (1971), who detected specific IgM in all patients up to 18 days after onset and in a proportion of patients up to 11 weeks after onset.

Radioimmunodiffusion after sucrose gradient fractionation is probably the most sensitive method for the detection of rubella-specific IgM (Ogra *et al.* 1971). These workers found specific IgM in all patients with rubella up to 1 month from onset and in the majority up to 2 months. They found it in only 1 of the 25 patients at 4 months, and none at 6–8 months, so that the test is not too sensitive. Unfortunately, the technique requires equipment more specialized than is found in most routine diagnostic laboratories.

The only workers to compare two methods are Forghani *et al.* (1973). They found all of 18 sera taken between 3 and 20 days after onset were positive by both sucrose density separation with HAI and by immunofluorescence. However, there was no correlation between the titres obtained by the two tests. They considered that immunofluorescence was a more difficult test to do than sucrose density

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separation. The simplified sucrose technique described here seems to be more sensitive than the techniques described by others except Ogra *et al.* (1971). It is comparatively simple and as many as 5 specimens can be tested per night.

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REFERENCES

BANATVALA, J. E., BEST, J. M., KENNEDY, E. A., SMITH, E. E. & SPENCE, M. E. (1967). A serological method for demonstrating recent infection by rubella virus. *British Medical Journal* iii, 285.

BAUBLIS, J. V. & BROWN, G. C. (1968). Specific response of the immunoglobulins to rubella infections. *Proceedings of the Society for Experimental Biology and Medicine* **128**, 206.

BEST, J. & BANATVALA, J. E. (1969). Serological tests for rubella. *Lancet* ii, 546.

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

BÜRGIN-WOLFF, A., HERNANDEZ, R. & JUST, M. (1971). Separation of rubella IgM, IgA and IgG antibodies by gel filtration on agarose. *Lancet* ii, 1278.

COHEN, S. M., DUCHARME, C. P., CARPENTER, C. A. & DEIBEL, R. (1968). Rubella antibody in IgG and IgM immunoglobulins detected by immunofluorescence. *Journal of Laboratory and Clinical Medicine* **72**, 760.

CRADOCK-WATSON, J. E., BOURNE, M. S. & VANDERVELDE, E. M. (1972). IgG, IgA and IgM responses in acute rubella determined by the immunofluorescent technique. *Journal of Hygiene* **70**, 473.

CRADOCK-WATSON, J. E., RIDEHALGH, M. K. S., BOURNE, M. S. & VANDERVELDE, E. M. (1973). Nasal immunoglobulin responses in acute rubella determined by the immunofluorescent technique. *Journal of Hygiene* **71**, 603.

DESMYTER, J. (1972). Absence of non-acute rubella IgM antibodies in chronic hepatitis and rheumatoid arthritis. *Lancet* ii, 1141.

DESMYTER, J., SOUTH, M. A. & RAWLS, W. E. (1971). The IgM antibody response in rubella during pregnancy. *Journal of Medical Microbiology* **4**, 107.

FIELD, P. R. & MURPHY, A. M. (1972). The role of specific IgM globulin estimations in the diagnosis of acquired rubella. *Medical Journal of Australia* **2**, 1244.

FORGHANI, B., SCHMIDT, N. J. & LENNETTE, E. H. (1973). Demonstration of rubella IgM antibody by indirect fluorescent antibody staining, sucrose density gradient centrifugation and mercaptoethanol reduction. *Intervirology* **1**, 48.

FRASER, K. B., SHIRODARIA, P. V. & STANFORD, C. F. (1971). Fluorescent staining and human IgM. *British Medical Journal* iii, 707.

GUPTA, J. D., PETERSON, V., STOUT, M. & MURPHY, A. M. (1971). Single-sample diagnosis of recent rubella by fractionation of antibody on Sephadex G-200 column. *Journal of Clinical Pathology* **24**, 547.

GUPTA, J. D., PETERSON, V. J. & MURPHY, A. M. (1972). Differential immune response to attenuated rubella virus vaccine. *Infection and Immunity* **5**, 151.

HAIRE, M. & HADDEN, D. S. M. (1970). Immunoglobulin responses in rubella and its complications. *British Medical Journal* iii, 130.

HAIRE, M. & HADDEN, D. S. M. (1972). Rapid diagnosis of rubella by demonstrating rubella-specific IgM antibodies in the serum by indirect immunofluorescence. *Journal of Medical Microbiology* **5**, 237.

HAIRE, M., ADAIR, B. MCC. & FRASER, K. B. (1972). The effect of temperature and time of incubation in the preparation of antigens for detection of virus-specific IgM antibody. *Clinical and Experimental Immunology* **13**, 327.

https://doi.org/10.1017/S0022172400042674 Published online by Cambridge University Press

NEWMAN, S., HORTA-BARBOSA, L. & SEVER, J. L. (1969). Serological tests for rubella. *Lancet* ii, 432.

OGRA, P. L., KERR-GRANT, D., UMANA, G., DZIERBA, J. & WEINTRAUB, D. (1971). Antibody response in serum and nasopharynx after naturally acquired and vaccine-induced infection with rubella virus. *New England Journal of Medicine* **285**, 1333.

PATTISON, J. R. & MACE, J. E. (1973). A simple, inexpensive gel filtration technique for use in diagnostic serology. *Journal of Clinical Pathology* **26**, 309.

PEAD, P. J. (1974). Estimation of rubella-specific IgM antibody in sera by single fraction collection on Sephadex G-200. *Medical Laboratory Technology* **31**, 159.

SEVER, J. L. (1969). Immunoglobulin determinations for the detection of perinatal infections. *Journal of Pediatrics* **75**, 1111.

VESIKARI, T. & VAHERI, A. (1968). Rubella: a method for rapid diagnosis of a recent infection by demonstration of the IgM antibodies. *British Medical Journal* i, 221.

https://doi.org/10.1017/S0022172400042674 Published online by Cambridge University Press