

## Reorienting gradient centrifugation in the determination of rubella specific IgM

R H CAVE *Public Health Laboratory, University Hospital, Queen's Medical Centre, Nottingham NG7 2UH, UK*

The laboratory diagnosis of acute or congenital rubella frequently rests on the presence of rubella specific IgM in serum.<sup>1,2</sup> Separation of serum IgM from IgG by sucrose density gradient centrifugation followed by the demonstration of haemagglutination-inhibiting activity in the IgM containing fractions is widely used for this.<sup>1</sup> With swing-out rotors centrifugation times of 16 hours or more are necessary. Use of reorienting sucrose gradients in a fixed-angle, vertical rotor allows this time to be considerably reduced. In this type of rotor, a sucrose gradient is gently accelerated, during which time it reorientates through 90° so that its length is the diameter of the centrifuge tube. Molecules then have a shorter distance to travel before adequate separation is achieved. The gradient returns to its original position when the rotor is decelerated. This report describes the use of such a rotor in the determination of rubella specific IgM.

### Methods

#### REORIENTING GRADIENTS FOR VERTICAL ROTOR

0.2 ml of a 1 in 2 dilution of untreated serum in DGV buffer was layered on to 4 ml of a 12.5-37.5% sucrose gradient in a 2 × ½ in polyallomer ultracentrifuge tube and overlaid with 0.2 ml of distilled water. Gradients were then centrifuged at 50 000 rpm (277 780 *g* at *R*<sub>max</sub>) for 1 hour 55 minutes in a Sorvall TV865 Ultravert rotor in a Sorvall OTD65 ultracentrifuge fitted with an acceleration rate controller.

#### CONVENTIONAL GRADIENT FOR SWING-OUT ROTOR

0.5 ml of a 1 in 5 dilution of untreated serum in DGV buffer was layered on to 4.5 ml of a 12.5-37.5% sucrose gradient and centrifuged at 40 000 rpm (192 000 *g* at *R*<sub>max</sub>) for 16 hours in a SW50.1 rotor in a Beckman L5-65 ultracentrifuge.

Twelve fractions of approximately 0.36 ml were collected after piercing the bottom of the centrifuge tube with a hypodermic needle. These were then

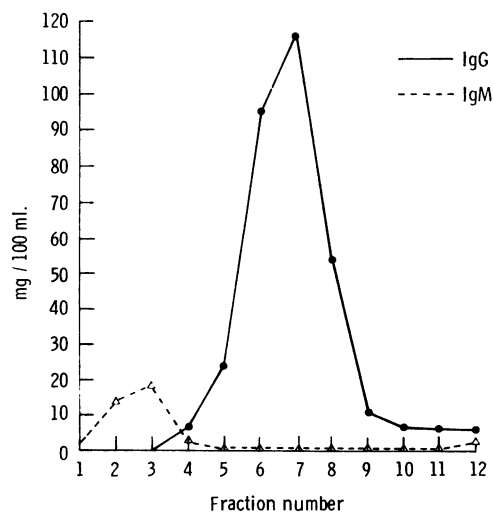
titrated for haemagglutination-inhibiting activity by a microplate method with 4 units of rubella haemagglutinin.<sup>3</sup>

Immunoglobulin concentrations were determined turbidimetrically on a Centrifichem model 400 (Union Carbide).<sup>4</sup>

### Results and discussion

Conditions were chosen, for the determination of rubella specific IgM by reorienting gradient centrifugation, that gave similar results when the same sera were tested by the conventional swing-out rotor method. Inconsistency in the location of the IgM was found to occur when higher speeds than 50 000 rpm were used in attempts to reduce to centrifugation time further. The efficiency of separation of total IgM from IgG, the ease of interpretation of the results, and the sensitivity were evaluated for the conditions given.

The Figure indicates the immunoglobulin concentrations in fractions recovered from a reorienting gradient. Resolution of the IgM and IgG bands was high, IgM being concentrated in fractions 2 and 3 in which there was no detectable IgG. Demonstration of rubella specific IgM is dependent upon the detection of HAI activity in these initial fractions. Typical results of HAI tests on fractions obtained by both the reorienting gradient method and the swing-out rotor method are given in the Table. For serum 1 the HAI activity present in fractions 2 and 3 by both methods indicates the presence of rubella specific IgM. In



*Immunoglobulin concentrations in fractions obtained from a reorienting gradient.*

*HAI titres obtained on two sera fractionated by both methods*

Rotor	Serum	Fraction No.											
		1	2	3	4	5	6	7	8	9	10	11	12
Vertical	1	—	8	16	8	32	≥ 64	≥ 64	≥ 64	32	16	32	32
Swing-out	1	—	4	8	8	4	32	≥ 64	≥ 64	≥ 64	16	16	32
Vertical	2	—	—	—	—	16	≥ 64	≥ 64	32	8	8	32	32
Swing-out	2	—	—	—	—	8	32	≥ 64	≥ 64	8	8	32	32

serum 2, no such activity is present and it is therefore negative. The close agreement between the two methods was found to be highly reproducible when the tests were repeated a further six times.

The capabilities of the methods in detecting low concentrations of virus specific IgM were compared as follows: Serial doubling dilutions of a rubella IgM positive serum were made using pooled, rubella IgM negative serum as the diluent. Centrifugation, fractionation, and HAI tests were carried out as described above. All dilutions gave identical results by both methods, the virus specific IgM still being detectable at a dilution of 1 in 8 of the original serum. Thus the two methods appeared to be of equal sensitivity.

Although the cost of a vertical rotor is only marginally more than that of a swing-out rotor, extra equipment for the slow acceleration phase is necessary for reorienting gradients. There is, however, an 80% reduction in centrifugation time. This, plus the provision for eight gradients in vertical rotors, allows tests to be done on as many as 16 sera in a single working day by the reorienting gradient method. The results appear comparable in accuracy to those obtained by the conventional swing-out rotor method.

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#### References

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Requests for reprints to: R H Cave, Public Health Laboratory, University Hospital, Queen's Medical Centre, Nottingham NG7 2UH, UK.



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R H Cave

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