specific as HRA has been reported.[5] Distinct antigenic differences between rotaviruses from different species, however, result in a variation of cross reactivity to these determinants and reflect reported[6] differing levels of sensitivity using antisera raised against human or animal rotavirus.

We conclude that HRA-latex is a quick, simple, and sensitive method of detecting rotavirus, particularly in a routine clinical laboratory without electron microscopy facilities.

TM JOHNSON
J SKINGLE
AP GILLETT
Department of Microbiology, Pathology Laboratory, Stoke Mandeville Hospital, Aylesbury, Buckinghamshire

References

[1] Moosai RB, Alcock R, Bell TM, *et al.* Detection of rotavirus by a latex agglutination test, Rotalex: comparison with electron microscopy, immunofluorescence, polyacrylamide gel electrophoresis, and enzyme linked immunosorbent assay. *J Clin Pathol* 1985:**38**:694–700.

[2] Sanekata T, Yoshida Y, Okada H. Detection of rotavirus in faeces by latex agglutination. *J Immun Methods* 1981:**41**:377–85.

[3] Grauballe PC, Vestergaard BF, Meyling A, Genner J. Optimised enzyme-linked immunosorbent assay for detection of human and bovine rotavirus in stools. *J Med Virol* 1981:**7**:29–40.

[4] Shirley JA, Beards GM, Thouless ME, Flewett TH. The influence of divalent cations on the stability of human rotovirus. (*Arch Virol* 1981:**67**:1–9.

[5] Sarkkinen HK. Human rotavirus antigen detection by enzyme-immunoassay with antisera against Nebraska calf diarrhoea virus. *J Clin Pathol* 1981:**34**:680–5.

[6] Yolken R, Wyatt RG, Kapikian AZ. ELISA for Rotavirus. *Lancet* 1977;ii:819.

Tests for detecting rubella specific IgM

Drs Cubie and Edmond recently reported their experience with five different tests to detect rubella specific IgM.[1] We tried a new enzyme linked immunosorbent assay (ELISA) kit for the detection of rubella IgM antibodies, which was prepared by the Division of Microbiological Reagents and Quality Control (DMRQC) of the Public Health Laboratory Service and compared it with Rubenz M II (Northumbria Biologicals Ltd). The protocols for these two assays were very similar. Both tests used a μ chain capture technique and monoclonal anti-rubella immunoglobulin conjugated to horseradish peroxidase. The objective of the trial was to seek advantages in terms of sensitivity and ease of use over the current method (fractionation on sucrose density gradient followed by haemagglutination inhibition).

Material and methods

Sera were selected on the basis of the rubella IgM titre by haemagglutination inhibition after fractionation: there were five categories (titres shown in parentheses); high ($\geq$64), intermediate (16–32), low (4–8), equivocal (2), and negative ($<$2). A serum with an IgM titre of 512 was diluted in negative serum to find the dilution at which it became non-reactive in each assay. Inadequate serum samples prevented repetition of the fractionation technique. The sucrose density gradient and haemagglutination inhibition method was refined to be as rapid, uncomplicated, and sensitive as possible. Discontinuous sucrose gradients were prepared in five steps (range 37·5%–12·5% w/v sucrose in dextrose gelatin veronal buffer), and 250 μl samples of serum were immediately layered on top and the gradients centrifuged at 35,000 rpm (125,000 $\times$ *g*) for 16–18 hours. The fractions containing IgM were titrated from 1/2 against 4 HAU_{50} of rubella haemagglutinin (DMRQC) with and without treatment with 2 mercapto ethanol (Sigma) for one hour at 37°C.

Rubenz M II was used in accordance with the manufacturer's instructions and differed from the test described by Cubie and Edmond in that phosphate buffered saline-Tween was used for the control well in place of "negative" antigen and that the cut off level for a positive result was $>$80% of the net value obtained with the 5 unit control serum. A reading of 50–80% of the 5 unit value was regarded as an equivocal result. The technique used in the DMRQC test has been described elsewhere.[2]

Plates were read on a Titertek Multiskan spectrophotometer, and in the case of the DMRQC assay results were interpreted by an interfaced BBC B microcomputer using Plateskan 2 software (IQ Bio Ltd) to plot the control sera (100, 30, 10, 3 arbitrary units) on to a standard curve, which calibrated the values of the test sera in arbitrary units (au). The Table shows the IgM values expressed in au of sera tested in the various categories. The test could be read reliably by comparing the visual or spectrophotometric absorbance values with the 10 au control serum used as a cut off. Values between 3 and 10 au were regarded as equivocal for the purposes of this trial.

The arbitrary unit standards for rubella specific IgM were originally established by Mortimer *et al* for the M-antibody capture radioimmunoassay (MACRIA)[3] and 3 units was taken as the cut off level. The DMRQC ELISA is based on the MACRIA but the DMRQC chose to use a nominal value of 10 au for the cut off level; hence all values of rubella specific IgM obtained with the DMRQC ELISA had to be divided by three for comparison with results of Rubenz M II, which uses units directly based on MACRIA standards.

Results

Accurate calibration of the Rubenz M II results was impossible with the control sera provided, and the format of the test (strips of 12 wells) did not lend itself to the structure of the computer program. Crude estimates of the unit measurement of samples were prepared from a manually drawn curve.

Eighteen sera were positive according to the criteria of both ELISAs. Of the remainder, seven were unreactive in the DMRQC assay, and a further six sera that gave equivocal values did not react in Rubenz M II. The estimated unit measurements of samples by Rubenz M II were lower by a factor of three than the results in au obtained with the DMRQC assay, but this was expected. These and the results of tests on dilutions of the strongly positive serum showed that the two assays had identical sensitivity, which was broadly equivalent to the sucrose density gradient and haemagglutination inhibition method. Of nine sera that gave equivocal results by sucrose density gradient and haemagglutination inhibition, one was reactive in both ELISAs and one equivocal in both. These values accorded with the eventual interpretation of the patients' rubella state, which had been reached on the basis of clinical information and reference tests performed elsewhere. Thus we concluded that there was no clear advantage to be obtained by the use of either assay.

Conclusion

With respect to the fractionation technique, no advantage was found (in terms of time and effort) for using the ELISAs over the sucrose density gradient and haemagglutination inhibition method. All three methods are designed to be set up one and a half to two and a half hours before the end

of the working day and to run overnight with a result obtained for each between three and five hours after the start of the next day. The amount of time required for each assay was not very different (80 to 100 minutes). Therefore, although the manual formation of sucrose gradients might be considered by some to be tedious, we doubt the opinion of Cubie and Edmond that "the time consuming and labour intensive nature, with resulting high cost, of the SDGHAI is familiar to most diagnostic laboratories."

In this laboratory sucrose density gradient and haemagglutination inhibition is completed within about 22 hours, and the testing of fractions for their haemagglutination inhibition activity is coincident with the routine testing of unfractionated sera for antibody to rubella virus, so that the same materials and equipment are used, making the procedure quicker. The capacity of the ultracentrifuge copes comfortably with the number of IgM estimations in seasons when rubella is not epidemic, and the maximum batch size ensures a run frequency that provides an undelayed result. Manufacturers of ELISA kits for rubella specific IgM currently recommend confirmation of positive results with a fractionation method, so retention of an ultracentrifuge or its equivalent is still desirable, at least in reference centres. Such equipment would undoubtedly have other uses in the laboratory; it will be years before whole serum IgM assays for all viral infections are commonly available. Hence cost analysis is complicated.

The sensitivity of sucrose density gradient and haemagglutination inhibition varies between laboratories, as will any test. Experience has shown that in this laboratory rubella IgM values of around 5–6 units (equivalent to 15–18 au in the DMRQC ELISA) are detectable by sucrose density gradient and haemagglutination inhibition, and the results of this trial confirm that view.

Techniques such as MACRIA are sensitive to minute amounts of IgM, which sucrose density gradient and haemagglutination inhibition at its best cannot detect, [3] although such sensitivity may pose problems of interpretation in some cases; nevertheless, these tests are more reliable for the diagnosis of congenital rubella.

As both ELISA kits tested were equally sensitive and neither had any clear advantages over the other, both would be suitable as supplementary assays to sucrose density gradient and haemagglutination inhibition for testing serum samples from neonates or other very small samples, or during rubella epidemics, using sucrose density gradient and haemagglutination inhibition to confirm positive results.

The continuing presence of an ultracentrifuge in this laboratory for the forseeable future is ensured by the need for confirmatory rubella IgM tests and for testing for IgM antibodies to other agents, so it will probably continue to be used for first line rubella specific IgM assays supplemented by an ELISA.

GM SUTEHALL
Clinical Microbiology and Public Health Laboratory, Addenbrooke's Hospital, Cambridge CB2 2QW

References

[1] Cubie H, Edmond E. Comparison of five different methods of rubella IgM antibody testing. *J Clin Pathl* 1985;**38**:203–7.
[2] Bellamy K, Hodgson J, Gardner PS, Morgan-Capner P. The Public Health Laboratory Service IgM antibody capture ELISA for the detection of rubella-specific IgM. *J Clin Pathol* 1985;**38**:1150–4.
[3] Mortimer PP, Tedder RS, Hambling MH, Shafi MS, Burkhardt F, Schilt U. Antibody capture radioimmunoassay for anti-rubella IgM. *J Hyg* 1981;**86**:139–53.

Arbitrary units of rubella IgM obtained with DMRQC ELISA and titres of haemagglutination inhibition IgM antibody in fractionated sera

Sucrose density gradient and haemagglutination inhibition (titre)	*DMRQC ELISA (au)*						
	No	*<3 (Negative)*	*3–10 (Equivocal)*	*10–20*	*30–40*	*40–50*	*>50*
High (≥64)	6						6
Intermediate (16–32)	7				1		6
Low (4–8)	4				1	1	2
Equivocal (2)	9	3	5	1			
Negative (<2)	6	4	2				

Separation of lymphocytes from peripheral blood: improvement of yields using buffy coat preparations

McKeating *et al* recently re-examined currently used methods of lymphocyte separation.[1] They confirmed that optimal yields are achieved by use of density gradients at room temperature throughout the procedure, a practice recommended by manufacturers (Lymphoprep Nyegaard, Oslo) and workers in the field.[2]

When we separated lymphocytes from whole blood by this standard method total cell recovery of 55 ((SD) 15)% and lymphocyte recovery of 45 (9)% occurred. This yield did not improve by including either supernatant plasma or the upper part of the density gradient during cell harvesting.[3] These yields have been consistent over the last year and are lower than those quoted by McKeating. The method is, however, subject to variability between laboratories because of the variable degree to which mononuclear cells adhere to different plastics.

We recently conducted a trial to compare standard methods with the use of buffy coats prepared from undiluted whole blood in an effort to improve yield and reduce the amount of density gradient required.

Buffy coats were prepared by centrifugation at room temperature at 100 × *g* for five minutes. Test tubes were then gently tilted to separate the buffy coats. They were removed by wide bore pipettes, diluted in 1 ml of Hanks's solution, and layered over an equal volume of lymphoprep at room temperature. Centrifugation was at 400 × *g* for 35 minutes. Aliquots of the initial blood sample and lymphocyte suspension recovered after three washes were analysed by the Technicon H6000 autoanalyser.

As far as we know such an accurate profile of cells recovered over density gradients has not been performed previously. Figs. 1 and 2 show a typical profile before and after lymphocyte recovery. Results using samples of edetic acid from 98 normal donors showed