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 SDGHA1 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 equiv-

alent 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 foreseeable 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.

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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
High (≥ 64)	6					6
Intermediate (16-32)	7				1	6
Low (4-8)	4				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

Whole blood				Mononuclear fraction			
FBC				FBC			
	5.73	$\times 10^9$	WBC		4.12	$\times 10^9$	WBC
	4.25	$\times 10^{12}$	RBC	L	0.00	$\times 10^{12}$	RBC
	12.3	g/dL	Hb	L	0.0	g/dL	Hb
L	35.8	l/l	HCT	L	0.0	l/l	HCT
	84.2	μ^3	MCV	L	0.0	μ^3	MCV
	29.0	pg	MCH	L	0.0	pg	MCH
	34.5	g/dL	MCHC	L	0.0	g/dL	MCHC
	14.8		RDW	L	0.0		RDW
	324	$\times 10^9$	PLT		198	$\times 10^9$	PLT
	8.3	μ^3	MPV		7.7	μ^3	MPV
	46.9		PDW		50.0		PDW
	0.27	%	PCT		0.15	%	PCT
DIFFERENTIAL				DIFFERENTIAL			
%	TYPE	$\times 10^9$		%	TYPE	$\times 10^9$	
58.5	NEUT	3.35		L 8.4	NEUT	.35	L
33.3	LYMP	1.91		H 82.1	LYMP	3.38	
5.0	MONO	.29		2.6	MONO	.11	L
1.2	EOS	.07		L 0	EOS	0	L
.4	BASO	.02		.4	BASO	.02	
1.6	LUC	.09		H 6.5	LUC	.27	H

Fig 1 "H6000" printout showing cellular distribution

WBC = white blood cells; RBC = red blood cells; Hb = haemoglobin; HCT = haematocrit; MCV = mean cell volume; MCH = mean corpuscular haemoglobin; MCHC = mean corpuscular haematocrit; RDW = red cell distribution width; PLT = platelets; MPV = mean platelet volume; PDW = platelet distribution width; PCT = platelet crit.

Table 1 Comparison of cell recovery by two methods (figures in parentheses are numbers %)

Method	Mean (SD) total cell recovery	Mean (SD) total lymphocyte recovery	Mean (SD) total monocyte recovery	Mean (SD) purity	No of samples
Standard	55 (15)	45 (9)	8.3 (4)	77 (4)	50
Buffy coat	76 (5)	64 (5)	16 (5)	77 (5)	98

Fig 2 "H6000" analysis of blood and mononuclear cell fraction
RBC = red blood cells; PLT = platelets; PEROX = peroxidase

edetic acid from 98 normal donors showed that we could improve our previous mean yield by 20% (Table). Viability of recovered cells was 98% and purity 77%. Interestingly, other workers found that a major proportion of unrecovered lymphocytes were lost in the buffy coat region of erythrocytes sedimenting through the density gradient.

Minor modifications to the Boyum method⁴ over 20 years have failed to produce a substantial improvement in yield. We believe that prior concentration of the mononuclear cells in whole blood by centrifugation is a new approach which will ensure a substantial improvement in the yield of lymphocytes and reduce reagent costs, as the

volume of separation medium required can be reduced by 80%. We have also been able to show that this method is equally effective for leukopaenic patients.

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Dr Markey and others reply as follows:

Not all workers in this field are separating lymphocytes from whole blood at room temperature. Some notable exceptions are mentioned in our recent letter; and we therefore reported our findings using different condi-