The steel finger spring facilitates rapid change of the catalyst as required.

In routine use the Whitley anaerobic jar has proved entirely reliable and has performed satisfactorily both with external cylindered gas and with gas provided internally from both GasPak and GasKit generators.

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A method for standardised resuspension of lymphocyte E-rosettes

R. C. POTTS, P. C. HAYES, W. KAY¹, A. J. ROBERTSON, AND J. S. BECK *Department of Pathology, University of Dundee, and Medical Physics Department¹, Ninewells Hospital and Medical School, Dundee, UK*

In recent years the number of requests to immunology laboratories for enumeration of T (thymus dependent) and B (bone marrow derived) lymphocytes in peripheral blood has increased considerably. T lymphocytes can be identified by their ability to form non-immune rosettes with sheep red blood cells (Fröland, 1972; Jondal *et al.*, 1972); although the enumeration of E-rosettes is relatively simple, the results may be invalidated by disruption during resuspension of the pellet. Since the widely used method of manual Pasteur pipette resuspension produces variable disruption, depending on the experience of the worker, various attempts at standardisation of the procedure have been proposed, such as the use of a Matburn rotary mixer (Papamichail *et al.*, 1972) or manual rolling of the tubes (Roszman *et al.*, 1977), but these have proved unsatisfactory in this laboratory. To overcome this technical problem we designed a simple and inexpensive piece of apparatus (Figure) which, regardless of the operator's experience in the technique, gives reproducible results.

Material and methods

APPARATUS

This consists of a synchronous motor and gearbox driving an arm bearing a free-running roller at 6 rpm within a U-shaped base plate. As the arm rotates, the roller has a peristaltic effect on plastic tubing which is open to the atmosphere at one end and at the other is connected to a Pasteur pipette held with a clip at a constant position within a plastic test tube (LP3, Luckham Ltd, Burgess Hill, Sussex) containing the cell pellet. During the first half of each cycle of the rotor, a standard volume of supernatant is slowly and gently drawn into the Pasteur pipette by compression of the tubing; the speed of rotation is such that during the second half

Figure Apparatus for resuspension of cell pellet.

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Table Comparison of E-rosette counts after manual and machine resuspension

Experiment	No. of laboratory staff participating	Percentage of peripheral blood lymphocytes forming E-rosettes after:							
		Manual resuspension				Machine resuspension			
		Mean	±	Standard deviation	Coefficient of variation	Mean	±	Standard deviation	Coefficient of variation
1	6	42.85	±	6.15	14.35%	44.85	±	1.47	3.28%
2	6	47.97	±	5.32	11.09%	48.10	±	4.34	9.02%
3	8	48.65	±	1.82	3.74%	48.94	±	0.77	1.57%

of the cycle the supernatant falls under gravity while the tubing is released.

LYMPHOCYTE SEPARATION AND E-ROSETTE DETERMINATION

For assessment of this apparatus, lymphocytes were obtained by Ficoll-Paque (Pharmacia, Uppsala, Sweden) gradient centrifugation of heparinised venous blood from one healthy donor. The method of E-rosette preparation was a modification of that of Stjernswärd *et al.* (1972). After resuspension, a minimum of 200 lymphocytes were counted in a haemocytometer: lymphocytes with 3 or more adherent sheep red blood cells were considered to be rosette-forming cells.

With the pump resuspension of the cell pellet is obtained in all cases when the specimen is given 10 cycles, with rotation of the tube through 90° on its long axis every alternate cycle. For a given cell pellet, the percentage of E-rosettes found after 8 cycles of resuspension remained unaltered with repeated cycles until the experiment was stopped after 20 cycles.

Results and discussion

We have compared T lymphocyte percentages after resuspension by laboratory staff of varying experience in handling these cells, using both the manual Pasteur pipette method and the apparatus described above. All samples were counted by the same individual whose observer error was known not to exceed 2% in repeated counts of one sample. The results of three experiments are shown in the Table. It was found that in each experiment, although the mean values were not significantly different using the two methods of resuspension, the coefficient of variation was appreciably lower with the resuspension apparatus. When the resuspension was performed by individuals little practised in this technique, the difference in coefficient of variation (13.33% manual: 5.58% machine) was particularly noticeable.

We have now used the apparatus for four months; it has proved a reliable method of standardising the most variable step in E-rosette preparation, enabling individuals of limited experience with the technique to produce reliable results. The apparatus has led to an increase in laboratory efficiency by allowing more specimens to be handled and by preventing technician fatigue while performing what is normally a boring repetitive task. The lower coefficient of variation found with this method of resuspension indicates that the technique is now more reproducible, thus allowing abnormal populations to be more easily identified. It is likely to prove valuable in the 'active rosette' technique in which the rosettes are smaller and less stable.

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