J. clin. Path. (1963), **16**, 168

Technical methods

Rapid whole-blood platelet counting using an electronic particle counter

ROBERT D. EASTHAM *From the Department of Pathology, Frenchay Hospital, Bristol*

APPARATUS

A Coulter electronic particle counter, model A with an orifice tube for the counter, aperture 70μ, was used, and saline, 0·85 g. NaCl per 100 ml. distilled water, filtered to remove most particles (M.P.C. stainless steel laboratory filter, grade No. 100)[1]. Flat-bottomed siliconed glass containers, 25 mm. internal diameter, are required.

METHOD

Blood is collected into E.D.T.A. (4 mg. of ethylene diamine tetra-acetic acid, dipotassium salt per 2·5 ml. blood, dried on the surface of the container). Blood, 0·2 ml., is added to 2·3 ml. saline in a siliconed glass container and mixed. Centrifuge for 20 seconds at 750 to 1,250 r.p.m. Within this range the speed is not critical, but the duration of spinning is critical. After less than 20 seconds there is too high a red cell count in the supernatant fluid, and after 30 seconds there is loss of platelets into the deposit, the larger platelets being lost first. Each centrifuge needs testing at various speeds within the range indicated. Rapid acceleration to the required speed is essential, and was obtained on the model used by temporarily switching to a higher stop for 10 seconds. Smooth slowing is also required. The particular centrifuge used when switched off at 1,000 r.p.m. ceased spinning about 40 seconds later. (Its radius was 14 cm., and the centrifugal force is proportional to the square of the radius.)

Carefully pipette off the supernatant fluid. Add 0·5 ml. of supernatant to 99·5 ml. filtered saline and mix. This diluted sample is counted in the counter at settings advised for each machine by Coulter Electronics Ltd. This particular machine and orifice tube, set at threshold 15, is used at aperture current setting 7 (A.C.S. 7) to count all particles 3·6μ^3 and above and at A.C.S. setting at 3 to count all particles 38·3μ^3 and above.

Received for publication 4 August 1962

[1]Adelphi Manufacturing Co. Ltd., 20/21 Duncan Terrace, London, N.1.

CALCULATION

(Count at 7 − blank at 7) − (count at 3 − blank at 3) = machine count.

The corrected machine count (corrected for coincidence) = machine count + $\left(\frac{\text{machine count}}{1{,}000}\right)^2 \times 0{\cdot}8575$.

(A simple correction chart can be rapidly drawn up.)

The whole blood platelet count = (corrected machine count × 5) per c.mm.

The volume of supernatant fluid used per count is adjusted so that the uncorrected machine count is not less than 20,000 and not more than 80,000, since the optimum results are obtained when the count falls around 50,000 per 0·5 ml. of fluid counted. The calculation is adjusted according to the dilution of initial supernatant.

RESULTS

The reproducibility of the machine count of platelet-rich plasma and of the proposed whole-blood platelet count method is shown in Table I.

It was found that by varying the speed and duration of centrifugation a supernatant fluid with a low red cell count could be obtained. Using siliconed glassware or a polystyrene container, the time of spin required could be greatly reduced, and the loss of platelets into the deposit was also greatly reduced. By reducing the height of the fluid column spun, using a flat-bottomed tube of wide diameter, the time of spin needed and hence the platelet loss was further reduced. By increasing the red cell population in the saline the platelet loss could be reduced to zero. When whole blood is centrifuged, the Bagnold force (Bagnold, 1954) comes into action, whereby on application of a shearing force to a suspension of particles they become charged in proportion to the number of times they collide with one another and repel one another. The lighter, smaller particles tend to rise relative to larger, heavier particles. Similarly when particles fall through a solution of an electrolyte in water, they become electrically charged and repel one another, the smaller, lighter particles tending to rise relative to the larger, heavier particles (Elton and Hirschler, 1954). Also, because of mutual repulsion, the heavier particles settle more slowly than in a fluid which does not conduct electricity.

These phenomena are utilized in the proposed platelet count method, as can be seen in Tables II and III. Table III shows the results of recovery experiments, in which platelet-rich plasma is added to whole blood in saline.

TABLE I

REPRODUCIBILITY OF PLASMA PLATELET AND WHOLE BLOOD PLATELET COUNTS

Preparation	*Mean of 12 Replicates (per cm.)*	*±2 S.D. (per cm.)*	*Coefficient of Variation (%)*
20 c.mm. platelet-rich plasma + 100 ml. saline	542,840	11,370	1·1
0·2 ml. blood + 2·3 ml. saline spun at 1,250 r.p.m. for 20 sec. 0·5 ml. supernatant + 99·5 ml. saline	242,300	24,120	4·98

TABLE II

EFFECTS OF COLUMN HEIGHT AND CELL DENSITY ON PLATELET LOSS

Whole Blood (ml.)	Platelet-rich Plasma (ml.)	Saline (ml.)	r.p.m.	Time (sec.)	Theoretical Count	Actual Count	% Difference
	0·2	9·8	2,000	30	507,420	424,700	−16·3
	0·1	2·4	1,750	20	507,420	446,420	−12
0·2		9·8	2,000	30	242,300	226,840	−6·4
0·1		4·9	1,750	20	242,300	232,790	−3·9
0·2		4·8	1,750	20	242,300	236,640	−2·3
0·2		2·3	1,250	20	242,300	242,300	0

TABLE III

EFFECTS OF COLUMN HEIGHT AND CELL DENSITY ON RECOVERY OF ADDED PLATELETS TO DILUTED BLOOD SAMPLES

Whole Blood (ml.)	Platelet-rich Plasma (ml.)	Saline (ml.)	r.p.m.	Time (sec.)	Theoretical Count	Actual Count	% Difference
0·2	0·2	9·6	2,000	30	749,720	681,550	−9·1
0·1	0·02	2·38	1,250	20	343,790	326,800	−4·9
0·2	0·02	2·38	1,250	20	586,090	565,050	−3·6
0·2	0·1	2·2	1,250	20	992,020	1,001,870	+1

TABLE IV

WHOLE BLOOD PLATELET COUNTS AFTER REMOVAL OF PLATELET-RICH PLASMA AND ITS REPLACEMENT BY PLATELET-POOR PLASMA

Slow-spun Plasma Removed (ml.)	Slow-spun Plasma Platelet Count (per c.mm.)	Volume Added of High-spun Plasma (ml.)	Platelet Count of High-spun Plasma (per c.mm.)	Whole Blood Platelet Count (per c.mm.)	Theoretical Whole Blood Platelet Count (per c.mm.)
Specimen 1 P.C.V. = 45% Initial blood volume = 2·5 ml.					
				284,570	
0·65	755,630	0·65	4,000	81,720	89,150
0·75	178,540	0·75	4,000	21,560	30,570
Specimen 2 P.C.V. = 43% Initial blood volume = 10 ml.					
				201,300	
1·0	615,770	1·0	174,000	157,120	150,410
1·0	426,180	1·0	174,000	112,930	129,650
1·0	312,330	—	—	102,560	112,350

When 10 ml. of fluid is used platelets are lost. There is still some loss when 0·1 ml. of whole blood is added to 2·4 ml. saline. Using 0·2 ml. whole blood with 2·3 ml. saline the recovery of platelets appears to be nearly complete. It is suggested that this result is due to the Bagnold force. These phenomena also explain why it is not possible to estimate the whole blood platelet count from plasma platelet counts, correcting for the packed cell volume, when the plasma is obtained either by simple sedimentation or by slow centrifugation. In the first instance, the plasma platelet count is too high due to the charges developed during sedimentation. In the second instance, at slow speeds (500 r.p.m.) very high plasma platelet counts can be obtained, especially in the upper layers of the plasma, *i.e.*, the reverse of trapping, due to the action of the Bagnold force.

The total platelet count, obtained by spinning 0·2 ml. of blood plus 9·8 ml. saline at 2,000 r.p.m. for 30 seconds and counting the supernatant fluid plus the platelet counts obtained by washing the resulting deposit, was found to be virtually the same as a single whole blood count by the proposed method. In recovery experiments with whole blood, replacing platelet-rich plasma volume for volume by high-spun, platelet-poor plasma, the theoretical and actual whole blood platelet counts by the method were in reasonable agreement (Table IV).

SUMMARY

A simple rapid method for the counting of platelets in whole blood using an electronic cell counter is described. The results obtained are accurately reproducible, and the initial preparation of blood samples takes less time than does a duplicate cell count by the machine on one such prepared sample, each such duplicate count taking not more than two minutes per specimen. Recovery experiments demonstrate the validity of the proposed method.

I am grateful to Dr. W. J. Jenkins, of the North-East Metropolitan Regional Blood Transfusion Centre, for advice and encouragement; to E. Morgan, F.I.M.L.T., for technical assistance; and to D. Goodchild, B.Sc., of Coulter Electronics Ltd., for much technical advice. I am especially grateful to Dr. C. T. Burch, Ph. D., F.R.S., Department of Physics, Bristol University, for his helpful advice and explanation of both electrophoresis and the Bagnold force.

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