Estimation of lymphocyte percentage and number on the Coulter Counter, Model S Plus Phase II

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SUMMARY The Coulter Counter Model S Plus Phase II provided precise measurements of lymphocyte percentage and count and carry-over was negligible. Lymphocyte percentage values agreed well with those from the stained blood film except when the percentage was high and in these circumstances the instrument gave underestimates. When making artificial mixtures with increasing lymphocyte count a progressive underestimation of the lymphocyte percentage was also noted.

The display of leucocyte volume distribution was found to be a useful attribute particularly when the instrument alerted the operator to “rejected” profiles. Rejection by the instrument proved to be a helpful function occurring in the myeloid leukaemias, erythroblastosis, and in some cases when the lymphocytes differed from normal—for example, glandular fever and chronic lymphocytic leukaemia.

When the leucocyte volume distribution is studied two cell peaks of lymphoid (lymphocytes and lymphoblasts) and myeloid (granulocytes, monocytes and precursors except blast cells) origin can be recognised.^{1–8} Size distribution analysis may be used to provide a simplified differential white count of lymphoid and myeloid categories. Such a differential is adequate for screening purposes^{9–11} and the results agree well with those from the stained blood film.^{5–7}

Initially attempts at size distribution analysis required complex analysis by computer.^{4,5} However it proved possible to devise simple analogue computing circuits to perform the calculations.^{6,12} The next development was the demonstration that lymphoid and myeloid cells could still be distinguished by size when much of their cytoplasm had been lysed.¹³ This made it possible to use automated instruments such as the Coulter Counter Model S to perform screening differentials.^{7,13} The latest generation of Coulter Counters, the Model S Plus Phase II, now incorporates an improved lytic system which produces better separation of the lymphoid and myeloid peaks.¹⁴ This paper assesses the suitability of the Model S Plus Phase II for estimating lymphocyte percentage and count.

Material and methods

Blood samples were collected in K₂EDTA 1.5 mg/ml and processed within 6 h of venepuncture. Wedge films were examined for uniform spreading of the leucocytes and 500 cell differentials were performed if the film was satisfactory. All types of leucocytes and nucleated red cells were included in the differential.

The Model S Plus Phase II was calibrated with 4C Plus II. This instrument estimates the percentage lymphocytes as the percentage of impulses derived from white cells which fall between 45 and 99 fl.¹⁴ The percentage lymphocytes can then be multiplied by the total white cell count to obtain the lymphocyte count. Electronic checks are made on the size distributions (see Results, leucocyte volume distribution curves) and if these are unsatisfactory the result is “rejected” and not printed out. The result, however, can still be obtained via the data terminal if it is of interest.

Results

PRECISION

This was estimated in the whole-blood and predilute modes by making 20 replicate measurements on various blood samples (Table 1). It can be seen that the CV increased as the lymphocyte percentage and count decreased. The lymphocyte percentage

Table 1 Precision, expressed as a coefficient of variation (CV), in the whole-blood (WB) and predilute (PD) modes

Sample	Mean		CV (WB)		CV (PD)	
	Lymph %	Count	Lymph %	Count	Lymph %	Count
1	7.30	0.83	6.44	5.66	6.04	7.65
2	23.70	1.36	2.77	4.46	4.01	7.52
3	25.30	2.16	2.89	2.81	2.59	3.14
4	77.80	51.99	—	—	1.07	2.90

Samples 1-4 were, respectively, from a preoperative patient, a case of secondary polycythaemia, a normal subject and a case of chronic lymphocytic leukaemia from whom it was not possible to obtain sufficient blood for whole-blood studies.

precision was much the same in the whole-blood and predilute modes but the lymphocyte count tended to be less precise in the predilute mode.

CARRY-OVER

This was studied by three consecutive estimations of lymphocyte counts (a_1 , a_2 , & a_3) on a blood with a high value followed by three consecutive estimations (b_1 , b_2 , & b_3) on a blood with a low value. Carry-over was calculated as $100 \times (b_1 - b_3) / (a_1 - b_3)$; the values of $a_1 \times 10^9/l$, $b_1 \times 10^9/l$ and carry-over (%) in three separate experiments were 26.5, 1.5, 0.8; 26.9, 0.6, 0.76; 55, 1.1, 0.74.

EFFECT OF DILUTION

The effect of dilution was studied by diluting packed cells with autologous plasma from a case of chronic lymphocytic leukaemia. Table 2 shows the mean lymphocytic percentage, and the mean lymphocyte count, and the number of parts of cells (out of a total of 10 parts by volume). It can be seen that the lymphocyte percentage decreases consistently as the number of parts of cells increases. Statistical analysis shows that the lymphocyte count data is linear (analysis of variance for deviation about regression using all five values at each dilution gives $F = 0.88$, $p > 0.05$ with 6 & 32 degrees of freedom) but that the line does not pass through zero (intercept = $1.14 \times 10^9/l$, Student's t test to compare intercept with zero gives $t = 5.52$, $p < 0.01$ with 38 degrees of freedom).

Table 2

No of parts of cells*	Mean lymphocyte percentage†	Mean lymphocyte count ($\times 10^9/l$)†
1	82.0	6.34
2	80.4	12.04
3	80.6	17.94
4	77.6	23.34
5	78.4	28.32
6	76.2	34.00
7	75.2	39.60
8	73.4	44.8

*Out of a total of 10 parts by volume.
†Five determinations

EFFECT OF STORAGE

Samples of blood from two patients with pseudo-polycythaemia were stored at 4°C and room temperature, making five determinations of the lymphocyte percentage, at various time intervals (Fig. 1). It can be seen that results are stable at either temperature for up to 24 h but that the values vary thereafter and many samples are "rejected" as having unsuitable leucocyte volume profiles.

Samples of blood were also studied after various periods of predilution in Isoton Plus (Fig. 2). It can be seen that the lymphocyte percentage decreased in the first hour but then increased again. As soon as the dilutions were made those from two of the five subjects were "rejected" and by 1 h four out of five were.

WHOLE BLOOD AND PREDILUTE MODES

Blood samples from 20 patients were studied five times in each of the modes and the mean values are plotted in Fig. 3. Excellent agreement is seen.

Fig. 1 Changes in lymphocyte percentage in two blood samples stored at 4°C or room temperature (RT)

Fig. 2 Changes in lymphocyte percentage when dilutions in Isoton II are stored at room temperature

COMPARISON WITH THE BLOOD FILM

The lymphocyte percentage results obtained on the Model S Plus Phase II were compared with those from the stained blood film. Figure 4a shows the

Fig. 3 Comparisons between results in the predilute and whole blood mode

results obtained with all patients except those in certain special categories whose results are given in Figure 4b. The overall agreement is good though there is a tendency for the Model S Plus Phase II to underestimate the high lymphocyte percentage values (Figure 4a), this feature being particularly evident with blood samples from younger patients (age < 7 yr) and those with chronic lymphocytic leukaemia or glandular fever (Figure 4b). However, the more discrepant results were flagged as "rejected" because the leucocyte volume distribution was unsatisfactory for analysis. In cases of acute myeloblastic leukaemia, chronic myeloid leukaemia and myelofibrosis the profiles were "rejected".

In order to compare the Model S Plus Phase II lymphocyte percentage results with those obtained by other methods based on leucocyte volume analysis the variance of the points about the regression line (Model S Plus Phase II result on film result) was calculated. This variance was found to be 12.7, a significant improvement on the value of 23.5 found when the leucocyte volumes were studied on a Coulter Counter Model S¹⁰ and an even greater improvement on the value of 33.2 found when whole leucocytes were sized on a Coulter Counter Model FN after saponin lysis.⁹

LEUCOCYTE VOLUME DISTRIBUTION CURVE

These are displayed on the visual display unit of the Model S Plus Phase II. Figure 5 shows a typical result from a normal subject, the lymphoid peak being separated from the myeloid by the trough at approximately 110 fl. This is the distribution to be expected with the partially lysed leucocytes which are sized on the Model S Plus Phase II. The lymphocyte percentage is estimated as the % cells found between 45 and 99 fl.¹⁴

Many electronic checks are made on the size distribution to ensure it is suitable for analysis. These include estimating frequencies at 64, 150 and 270 fl and ensuring that at least one of these frequencies is greater than $3.3 \times$ frequency at 45 fl (—that is, no excess erythroblasts which, when lysed, appear near 45 fl) and greater than twice the frequency at 99 fl (— that is, good lymphoid/myeloid separation) and greater than $6.7 \times$ frequency at 450 fl (— that is, adequate lysis since non-lysed cells appear near 450 fl). If the size distribution does not meet the required specifications it is "rejected."

Examples of how the system of analysis operates are shown in Figs. 6 and 7. In children age < 7 yr, glandular fever and chronic lymphocytic leukaemia the lymphoid peak can be clearly seen (Fig. 6 a–c) but in a number of instances it is larger than usual and then the instrument flags the sample as "rejected". Problems also arise when large numbers of nucleated

Fig. 4 Comparison between automated results from the Model S Plus Phase II and those from the blood film
(a) results for the majority of hospital patients: ○ = samples rejected, ● = samples not rejected.
(b) results from patients in certain special categories:

	Rejected	Not rejected
Age < 7 yr	○	●
Glandular fever	□	■
Chronic lymphocytic leukaemia	◇	◆
Nucleated red cells	⊗	⊗

aml = acute myeloblastic leukaemia, cml = chronic myeloid leukaemia, mf = myelofibrosis

red cells circulate (Fig. 6d) since the lower part of the lymphoid profile may be obscured.
Myeloid abnormalities such as neutrophil leucocytosis and eosinophilia do not present any difficulties (Fig. 7 a, b) though the appearance of two myeloid peaks was an irregular feature in

eosinophilia. In acute myeloblastic leukaemia the myeloblasts appeared as a single peak larger than lymphocytes and the profile was “rejected” (Fig. 7c) whilst in chronic myeloid leukaemia the lymphocyte peak was obscured and the profile also “rejected” (Fig. 7d).

Fig. 5 Leucocyte volume distribution from a normal subject

Fig. 6 Leucocyte volume distributions from children age < 7 yr (A), patients with glandular fever (B), chronic lymphocytic leukaemia (C) or circulating normoblasts (D). The profile in D and the profiles numbered 2 in A–C were “rejected”.

Fig. 7 Leucocyte volume distributions from patients with neutrophil leucocytosis (A), 2 with eosinophilia (B), acute myeloblastic leukaemia (C) and chronic myeloid leukaemia (D). The profile in C–D were “rejected”.

Discussion

The Coulter Counter Model S Plus Phase II derived lymphocyte percentage agrees with the stained blood film better than estimates from the Coulter Counter Model S¹⁰ presumably because of improvements in the lytic reagent. The Model S derived results were themselves better than those on whole leucocytes⁶ because distinction between lymphoid and myeloid cells is improved by leucocyte lysis.

With earlier systems some overlap from lymphoid peak into myeloid was always observed and allowance was made for this in the calculation of the lymphocyte percentage.^{5, 6, 12} This prevented one problem which appears to have arisen with the Model S Plus Phase II, namely a relative underestimation of lymphocyte percentage when the lymphocyte count is high, a phenomenon observed in this study whether

the high count was due to disease or due to making artificial mixtures of cells and autologous plasma (see Results, Effect of Dilution). The likely explanation for the underestimation is that as the lymphocyte count increases the lymphocyte peak becomes wider and more cells exceed the 99 fl threshold and are counted as myeloid.

Presentation of the leucocyte volume distribution on the visual display unit of the instrument was found to be a very valuable feature, particularly when the profiles were “rejected”. Rejection alerted the user to the possibility of various abnormalities—for example, myeloid leukaemias, erythroblastosis, and some cases of glandular fever and chronic lymphocytic leukaemia. Cases of glandular fever and chronic lymphocytic leukaemia which were not rejected were, of course, recognisable by their high lymphocyte percentage and counts.

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