

Abridged differential leucocyte counts provided by a Coulter Channelyzer in a routine haematology laboratory

PATRICIA A. WYCHERLEY AND M. J. O'SHEA

From the Department of Pathology, Shrewsbury Hospital, Shrewsbury SY3 8XH, UK

SUMMARY We have used automated differential leucocyte counts by volume analysis for the past six months after initial evaluation had shown several advantages—namely, accuracy, simplicity, speed, and low cost. We describe the operation of the Coulter Channelyzer Model C-1000, which is linked to a Coulter Model S cell counter, analyse the cost, and comment on the clinical and laboratory consequences of adopting this method.

Automated procedures for blood counts would rapidly increase work output were it not for the delay caused by manually performed differential leucocyte counts. The purchase of instruments for this purpose is ruled out in the prevailing economic circumstances. A less costly alternative is to attach a Coulter Channelyzer to a Coulter cell counter. Models in the B, F, Z, or S series are suitable.

The Channelyzer analyses pulses from the cell counter on a height distribution basis and automatically sorts them into 100-size groups while the cell counter is actually counting. The volume of granulocytes and monocytes is about twice that of lymphocytes. Therefore bimodal distribution curves are produced on the Channelyzer's oscilloscope screen with normal or near normal bloods, and these two curves relate to the number of myeloid cells (neutrophils, eosinophils, and monocytes) and lymphocytes respectively. The Channelyzer also provides a numerical display of the number of cells represented by both curves or by the individual curves. From these data the percentages of myeloid cells and lymphocytes can be calculated.

The Channelyzer was not designed to be used with the Model S, but if its signal cable is attached to one of the Pre Amp cards satisfactory results are obtained. The Coulter S seems particularly well suited for this purpose. It provides standardisation of leucocyte dilution, of the volume of lysing agent added, and of the lysis time. Without this degree of standardisation there could either be altered

leucocyte volumes as a result of too large a volume of lysing agent or too long a lysis time, or both, or, conversely, incomplete destruction of the red cells, causing modifications to the leucocyte distribution curves.

Method

COULTER S AND CHANNELYZER OPERATION

Initially the Channelyzer requires calibration with a Coulter Model Z. The Model S lacks the comparable degree of adjustment that can be made to threshold level, gain, and aperture current. The Channelyzer must then be used with the signal input adapter switch in the Z position.

Channelyzer settings are used as shown in Table 1. With the count control switch and the count range switch in the positions indicated well-defined distribution curves are obtained. Accumulation in all channels will stop when the Coulter S has finished counting. However, if leucocyte numbers are sufficiently increased, especially those of one cell type, accumulation will cease as soon as there are 400 cells in any channel.

With a base channel threshold of 10 electronic noise and red cell debris are excluded. As indicated in Table 1, the window is left wide open so that most of the pulses within the Coulter S threshold limits are included in the Channelyzer's 100 channels. With the read-out switch in the 'integrate' position the upper and lower channel settings are defined by a bright marker, and when the 'integrate' switch is pressed the total number of cells between these lower

Table 1 *Channelyzer settings*

Control	Setting
Count control switch	Stop at full scale
Count range switch	400
Base channel threshold control	10
Window-width control	100
Lower channel switch	3
Upper channel switch	95, or where myeloid curve touches baseline
Read-out switch	Integrate

and upper channel settings is shown on the digital display.

We feed samples to the Coulter S at a maximum rate of 100 blood specimens every hour. One technician operates the Coulter S. A second technician manipulates the Channelyzer, as follows, and checks the printed-out results from the Coulter S.

- (1) Before each diluted and lysed sample reaches the counting bath the 'reset' and 'start' buttons are pressed.
- (2) Immediately the distribution curves are complete (which is simultaneously with the completion of the Coulter S counting period) the upper channel switch is turned to the channel representing the trough between the two curves. This channel rarely changes for normal or near-normal blood. The 'integrate' button is pressed and the number of cells in the lymphocyte curve is read off from the digital display and entered into a pocket calculator.
- (3) Then the upper channel switch is turned to the channel corresponding to one in which the myeloid curve touches the baseline; the 'integrate' button is pressed again and the total number of cells represented by both lymphocyte and myeloid curves is read off and entered into the pocket calculator. From these two sets of figures the lymphocyte percentage is calculated and noted down on the back of the Coulter form when it is extracted from the Coulter S printer.
- (4) The 'reset' and 'start' buttons must be pressed before the next sample reaches the counting bath.

A simple computer attached to the Channelyzer (there is a suitable output for this) would eliminate the manual stages outlined above. However, with some abnormal bloods it would be necessary to select the 'trough' channel manually if the trough had moved from its normal position.

EXAMINATION OF BLOOD FILMS

A film is scanned and, provided there are no leucocyte abnormalities or normoblasts present, the lymphocyte percentage from the Channelyzer is

recorded on the Coulter form. The neutrophil percentage is obtained by subtraction and is also recorded after the necessary adjustment for eosinophils or monocytes present has been made. Red cells and platelets are also assessed but a 100-cell differential count is omitted. This reduces our previous average examination time of three minutes per film by a conservative 30%.

Results

TYPES OF LEUCOCYTE DISTRIBUTION CURVE

The curve in Fig. 1 is typical of a normal bimodal distribution. Note that the lymphocyte curve spans only about 15-18 channels. Lymphocytes after treatment with Lyse S appear to have a fairly uniform volume. However, England *et al.* (1975), using pure preparations of lymphocytes, observed that some blood specimens contain a small population of lymphocytes whose volume is equivalent to that of the median neutrophil volume. The results with lymphocyte preparations counted in this laboratory did not agree with this finding (an example is shown in Fig. 2).

The myeloid curve extends across about 35 channels; its broad base is accounted for by monocytes which, because of their relatively larger volume are found in the higher channels. Figure 3 is a curve from a case of eosinophilia. It is indistinguishable from a normal curve and eosinophilia cannot be identified without examination of the blood film. Curves produced from preparations of myeloid cells do not indicate that any myeloid cells are as small as the median lymphocyte. The findings of England *et al.* (1975) were in agreement with this.

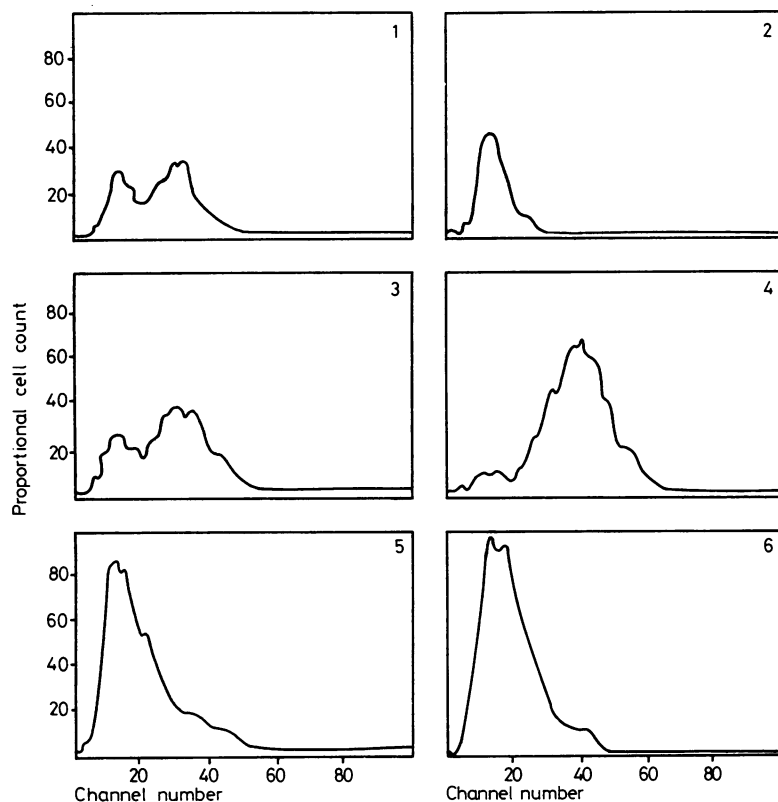
Abnormal blood specimens produce changes in the distribution pattern, and in some instances the bimodal pattern is replaced by a single peak (Figs 4-6). The presence of abnormal cells—for example, blast cells—sometimes results in lymphocyte precursors augmenting the myeloid curve or micro-myeloblasts being included in the lymphocyte curve. In these cases the Channelyzer's numerical read-out could not be used as a basis for a differential count.

COMPARISON OF VOLUME ANALYSIS AND VISUAL DIFFERENTIAL COUNTS

During a normal working week over 1200 specimens were checked by both methods. Agreement was found in 90% of counts. Generally, when they differed a second assessment of the blood film indicated that the volume analysis results were more correct and that cell distribution in the blood film was irregular. In only 2% of cases was a visual differential count necessary. These were cases with an eosinophilia or cases of leukaemia. Experience

Table 2 Representative examples of automated differential leucocyte counts by volume analysis from which the distribution curves shown in Figs 1-6 were constructed

Figure No.	Example	Leucocytes (%)				
		Neutrophils	Lymphocytes	Monocytes	Eosinophils	Atypical mononuclear cells
1	Normal blood	69	23	7	1	—
2	Pure lymphocyte preparation	—	100	—	—	—
3	Eosinophilia	31	31	1	37	—
4	Pyogenic infection	84	12	4	—	—
5	Infectious mononucleosis	16	60	6	—	18
6	Chronic lymphatic leukaemia	12	88	—	—	—



Figs 1-6 Leucocyte volume distribution curves illustrating examples shown in Table 2.

with varied clinical material over a six-month period has confirmed these findings. Hughes-Jones *et al.* (1974) when comparing the two methods did not obtain very good agreement with lymphocyte counts, although they were very satisfied with the myeloid cell counts. They believed that the smaller number of lymphocytes present resulted in a larger sampling error. Their lymphocyte values for the ratio of volume analysis to manual counts were between 0.6 and 1.5, with a few exceptions as wide apart as 0.2 and 4.4. Our range was 0.75-1.3; 0.4% of blood specimens lay between 1.4 and 2.7 (leukaemia excluded).

AGEING AND REPRODUCIBILITY TESTS

Our laboratory serves a district of 349 000 hectares with a population of 360 000. Postal specimens may be 48 hours old before they are tested. To assess the changes which might occur over this period blood specimens were put through the Coulter S/Channelyzer one hour after collection and then again after 24 and 48 hours. The distribution curves remained essentially unchanged.

Instrument precision was investigated using replicate studies. Blood specimens with lymphocytes ranging from 8% to 52% were resampled 15 times. The standard deviation for all the series was 1.1 ± 0.1

and the precision with which the instrument measured was 4% for normal lymphocyte counts, increasing to 11% where the count is low.

COSTING

The introduction of budgetary control and the current financial stringencies have emphasised the importance of a cost analysis before purchase of new equipment. The cost advantage of a Channelyzer lies in the saving of technician time, otherwise spent on differential counts. Our savings for 1976/7 on 40 000 differential counts equated to one minute per count. Based on a notional technician salary cost of £2.08 per hour the following cost saving is derived:

40 000 differential counts × 1 minute of time saved
60 minutes = 1 hour
× £2.08 hourly rate = £1868 per annum.
Current cost of machine = £4012.

Discussion

The availability and high cost of machines capable of automated differential counts led to an appraisal of another possible alternative to current practice. The Channelyzer system eliminates distributional errors associated with the preparation of blood films and also statistical errors, since well over 1000 cells are counted. However, an error will be introduced, because even careful selection of the trough channel leaves some overlap of the cell populations. Analysis of a number of tracings shows this to be always less than 6% of either lymphocyte or myeloid cells. Since the overlap is to either side of the trough it is always in part compensated and the final error is small compared with what can be produced with manually performed differential counts. The procedure is simple and uses an already well-established instrument, and no additional operator is needed. It goes part-way to fulfilling the criteria for an 'ideal automatic differential leucocyte counter' as outlined by Bentley and Lewis (1977).

Some beneficial clinical as well as laboratory consequences derive from this system. Blood count reporting in general is speeded up and since the white cell ratio is obtained along with the leucocyte count an immediate result can be given in out-patient cases. This is particularly useful for radiotherapy

clinics. The myeloid/lymphocyte percentages are available in cases in which a differential would not normally be recorded.

None of our laboratory technicians has had any problems in completing this work during the Coulter S cycle. Provided two carry out the Coulter S/Channelyzer operations—and that has always been our routine procedure for batches of work—the throughput is no slower than without a Channelyzer. The blood films are still examined but with a change of emphasis towards comments on abnormal findings. Experience with this practice over the past six months has confirmed the saving in technician's time and relief from the tedium of routine counts.

Our cost-saving calculations show that we can expect to pay for this equipment in two years and that thereafter we should make an annual saving of one-half a technician's salary. We shall be able to monitor this by examining the cost of a unit of 'weighted patient requests' year by year. The budget system allows the use of approved virement accounting—for example, approved reallocation of funds between items of expenditure—and it has been these factors which led to the availability of funds to purchase a Channelyzer at a time of strict financial control.

We think that with this method we have a useful stepping stone until the problems associated with fully automated procedures are resolved. There would remain the inevitable high cost of such instruments, and many laboratories would need to consider the more practical alternative assessed here.

References

- Bentley, S. A., and Lewis, S. M. (1977). Automated differential leucocyte counting: the present state of the art. *British Journal of Haematology*, **35**, 481-485.
- England, J. M., Bashford, C. C., Hewer, M. G., Hughes-Jones, N. C., and Down, M. C. (1975). Simple method for automating the differential leucocyte-count. *Lancet*, **1**, 492-493.
- England, J. M., Down, M. C., Bashford, C. C., and Sabry-Grant, R. (1976). Differential leucocyte-counts on Coulter Counter Model S (Letter). *Lancet*, **1**, 1134-1135.
- Hughes-Jones, N. C., Norley, I., Young, J. M. S., and England, J. M. (1974). Differential white cell counts by frequency distribution analysis of cell volumes. *Journal of Clinical Pathology*, **27**, 623-625.