A comparative study for the enumeration of peripheral blood white cell counts below $2.0 \times 10^9/l$ using counting chambers and the Coulter Counter Model 'S'

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SUMMARY Low white cell counts are performed in many laboratories by a visual method as the method of choice. In this study, the visual count has been compared to that obtained by the Coulter Model 'S'. The results obtained by the two methods show very close correlation, thus allowing the Coulter Model 'S' to be used with confidence for all low white cell counts provided certain precautions are taken.

In the evaluation of the Coulter Model 'S' by Barnard *et al.* (1969) and Brittin *et al.* (1969) measurement of the white count correlated well with visual techniques. Later work by Pinkerton *et al.* (1970) tested the linearity of the instrument using serial dilutions and reported no deviation in the white cell range of 1000 to 50 000 per μl . However, a comparison of Coulter Model 'S' and visual techniques for low white cell counts does not appear to have been attempted. In this series, the visual count has been compared to that obtained on the Coulter Model 'S' for all routine samples that gave a printout value for WBC of $2.0 \times 10^9/l$ or less.

The patients whose blood was tested had a variety of malignant conditions including Hodgkin's disease, breast carcinoma, teratoma testis, non-Hodgkin's lymphoma, and acute myeloid leukaemia. Most were having intensive chemotherapy or radiotherapy which was responsible for the low white cell count.

Methods

White cell counts were performed on 101 sequestrene samples by both the visual method and the Coulter 'S'.

VISUAL COUNT

A 1:10 dilution of blood in white cell counting fluid (2% acetic acid coloured with crystal violet) was

introduced into both sides of an Improved Neubauer counting chamber. The cells in at least 10 squares, and often all 18 squares, were counted by an experienced observer and the result was expressed as the count $\times 10^9/l$.

COULTER MODEL 'S'

The blood sampling valve was cleared of blood by aspirating Isoton several times. The instrument was cycled three to four times with Isoton and the blank reading was noted on the final cycle. The blood sample was introduced and the instrument cycled twice. On the second cycle the white cell printout was noted. The blank was subtracted from the printout value to obtain the corrected white cell count.

Results

The Figure shows all the results in the form of a scatter diagram. The number of cells counted for the visual count varied from 5 to 162.

The Coulter 'S' blank was usually in the range $0.2-0.5 \times 10^9/l$, only rarely going outside this range. Expressed as a percentage of the white cell printout value, it was usually below 40% but reached 80% on three occasions.

The scatter diagram shows a very high level of agreement between the two methods, and the calculated value for $r = 0.95$.

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Figure Scatter diagram of the 101 visual and Coulter 'S' results showing the line of equality between the two methods.

Discussion

Two factors affecting the count in the Coulter 'S' are bubble rates in the aperture bath and 'carry-over' from the previous sample. The first can be minimised by correct adjustment, and the second, which should not exceed 3% in any case, is avoided by first cycling with Isoton and then only noting the count when the blood sample is presented the second time.

The Coulter 'S' counts a much larger sample of cells than does a microscopist. For every $1.0 \times 10^9/l$ white cells in the whole blood, 100 cells are counted in the visual method while the Coulter 'S' counts over 2000 cells. This is derived as follows: The Coulter 'S' dilutes $44.7 \mu l$ blood in 10 ml Isoton = 1:233.7.

On addition of 1 ml Lyse S the dilution becomes = 1:246.1. At 6 inch Vacuum, $3 \times 100 \mu$ orifices sample 0.5 ml which contains

$$\frac{1000 \text{ (cells/10}^9\text{l)}}{246.1 \text{ (dilution)}} \times 500 \text{ (sample)} = 2031 \text{ cells.}$$

Thus from sample size alone the Coulter 'S' would

be expected to be more precise. Also in its favour is the fact that the counts from the three orifices are calculated and compared, and only if they agree within the 3 SD limits are they accepted. The close agreement of the Coulter 'S' and visual counting of small concentrations of blood leucocytes is additional evidence for the accuracy of both methods as performed in this study.

The imprecision of low visual counts due to the high percentage coefficient of variation is well known and understood. However, the best estimate of the true count by this method is still calculated from the actual number of cells counted, and in this study the two methods as usually performed are being compared.

If at the lower end of the range wide variations had been obtained due to the small sample size of the visual count, the scatter diagram would be expected to broaden out and this has not happened.

Therefore it appears that provided the method of operating described is employed, the Coulter 'S' can be used with confidence for low white cell counts.

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References

- Barnard, D. F., Carter, A. B., Crosland-Taylor, P. J., and Stewart, J. W. (1969). An evaluation of the Coulter Model S. *Journal of Clinical Pathology*, **22**, Supplement (Coll. Path.), 3, 26-33.
- Brittin, G. M., Brecher, G., and Johnson, C. A. (1969). Evaluation of the Coulter Counter Model 'S'. *American Journal of Clinical Pathology*, **52**, 679-689.
- Pinkerton, P. H., Spence, I., Ogilvie, J. C., Ronald, W. A., Marchant, P., and Ray, P. K. (1970). An assessment of the Coulter Counter Model 'S'. *Journal of Clinical Pathology*, **23**, 68-76.