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Technical methods

Rapid method for counting reticulocytes

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Among the several labour-saving methods for counting reticulocytes reviewed by Dacie and Lewis (1963) the least laborious is the Miller count described by Brecher and Schneiderman (1950). For this method the Miller graticule, a microscope eyepiece graticule with a square of side 7 mm. with a square of one ninth the area in one corner, is used and consecutive fields are counted, reticulocytes in the large square and all red cells in the small square. If x is the number of red cells counted in the small square, p is the proportion of reticulocytes and S_p is the standard error of p.

$$\text{Percentage of reticulocytes} = \frac{100 \times \text{reticulocytes in large squares}}{9x} \quad (1)$$

$$S_p = \sqrt{\frac{p(1 + 9p)}{9x}} \quad \ldots\ldots\ldots\ldots\ldots\ldots (2)$$

For the Miller count the product $9x$ is made approximately equal to 2,000 by counting 20 fields of a well-packed film at a total magnification of $\times$1,000. If, however, the product $9x$ is made equal to 1,000 the labour of counting is halved, the restrictions of well-packed films, and a total magnification of $\times$1,000 are removed and the percentage of reticulocytes can be calculated mentally.

$$\text{Percentage of reticulocytes} = \frac{\text{reticulocytes in large squares}}{10} \quad \ldots\ldots (3)$$

For the proposed quick method, the rapid Miller count, evenly spread films are counted at a magnification of $\times$1,000 or more. Reticulocytes are counted in large squares and all red cells in small squares and counting is continued till 111 red cells have been counted in small squares. The number of reticulocytes corresponding to 111 red cells is then estimated, thus making the product $9x$ equal to 1,000, and the percentage of reticulocytes is calculated from equation 3. Theoretical standard errors for this method are given in Table I with theoretical standard errors for the standard count, in which 1,000 cells are counted, and the Miller count (Brecher and Schneiderman, 1950).

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TABLE I

THEORETICAL STANDARD ERRORS OF STANDARD, MILLER, AND RAPID MILLER COUNTS

	Percentage of Reticulocytes			
	2	5	10	20
Standard count	0·44	0·69	0·95	1·26
Miller count	0·34	0·60	0·98	1·68
Rapid Miller count	0·49	0·85	1·38	2·37

EXPERIMENTAL

Twenty slides were prepared by a tube method (Dacie and Lewis, 1963) from each of four blood samples. A rapid Miller count was made with each slide and each count was continued as a Miller count, by continuing till approximately 222 red cells had been counted in small squares. A total magnification of $\times$1,250 was used. No difficulty was found in estimating the number of reticulocytes for the rapid Miller count. The number of red cells counted for the Miller counts varied from 218 to 226. The results are given in Table II.

DISCUSSION

Brecher and Schneiderman (1950) compared the Miller and standard counts and showed that the Miller count gave average values in agreement with the standard count and errors close to predicted values. The results in Table II show that the rapid Miller count also gives errors close to predicted values and agreement with the Miller count is close. Whether the accuracy of the rapid Miller count is adequate is a matter of opinion. As it is as accurate as the standard count for reticulocyte counts of 2% and less, and as the upper level of normal for the reticulocyte count in adults has been given as 2% (Whitby and Britton, 1963), it is adequate for screening normal adults. It is considered to be accurate enough for the detection of a reticulocyte response to treatment and for all diagnostic problems. In certain circumstances the error will be too large, both the Miller and standard

TABLE II

EXPERIMENTAL COMPARISON OF MILLER AND RAPID MILLER COUNTS IN FOUR SETS OF 20 COUNTS

	Miller Count	*Rapid Miller Count*	*Miller Count*	*Rapid Miller Count*	*Miller Count*	*Rapid Miller Count*	*Miller Count*	*Rapid Miller Count*
Average reticulocyte (%) of 20 counts	1·33	1·38	4·99	4·91	16·3	16·7	25·1	24·8
Observed standard error	0·24	0·34	0·50	0·60	1·0	1·4	2·6	3·4
Theoretical standard error	0·27	0·39	0·60	0·85	1·4	2·0	2·0	2·9