The interrelationships and stability of Coulter S-determined blood indices

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SYNOPSIS The blood of 104 medical inpatients was examined at various intervals during storage for 72 hours using a Coulter S counter. Over this period remarkable stability of the white cell count, red cell count, haemoglobin, and mean cell haemoglobin was demonstrated. This permits a useful interpretation of indices obtained in routine postal samples sent to the laboratory by general practitioners for screening. Furthermore, the implications of high correlations between red cell indices in freshly examined blood are considered and the regression line $MCV = 2.5 MCH + 16$ is derived. Using this relationship the normal range of the Coulter S MCHC is defined as 32.6-33.7 g/dl. The significance of the Coulter MCHC in present-day practice is reassessed and the importance of recognizing values for MCV and MCH not coinciding with the regression line is briefly discussed.

With the introduction and widespread use of the Coulter S counter, rapid, accurate and reproducible measurements of red and white cell numbers, mean cell volumes, and by computation the haematocrit, mean cell haemoglobin, and mean cell haemoglobin concentration have become readily available. However, the diagnostic significance attached to the values of these indices in routine haematological practice appears to merit careful reappraisal. Previous assumptions have been based on the inherently inaccurate 'manual' techniques involved in cell counting and haematocrit estimation. Though the errors of using haematocytometers have long been appreciated (Berkson, Magath, and Hurn, 1940), the advent of electronic particle counting as exploited in the Coulter counter has further revealed that cell numbers are consistently overestimated in counting chambers (Mattern, Brackett, and Olson, 1957). Recently, reinvestigation of manually derived haematocrits has emphasized the errors and inconsistencies due to plasma trapping between red cells of differing shapes and sizes (England, Walford, and Walters, 1972).

With the centralization of laboratory services, the ever-increasing demands made upon them by both hospital and general practitioners, and moves toward

'well population' screening, it is likely that blood analyses will be undertaken on samples with increasing 'dead times', ie, the intervals between venesection and analysis. Included in the annual workload of this laboratory are 25 000 postal samples submitted for routine blood examination by general practitioners throughout the North East of Scotland, the Orkney and Shetland Islands. It is thus of some immediate concern to us and, we hope, of more general interest to assess the effects of storage upon Coulter S-determined blood indices.

The study presented here therefore has two aims: to consider the interrelationships between Coulter S indices in fresh blood and to determine how the values of these indices change with time.

Materials and Methods

During a five-week period, venous blood samples were taken from 104 'medical' inpatients suffering a wide variety of disease (infections, uraemia, jaundice, cardiac failure, collagen disorders, anaemias, and leukaemias). The samples were divided equally into 0.5 dl bottles containing 1.2 g/l of dipotassium EDTA and stored at room temperature. Each daily batch of samples was processed with the routine work load of the laboratory at two, six, 24, 48, and 72 hours after venesection, a single analysis being made on each sample after mixing on a rotating rocker for 15 minutes. Throughout the study period the counter was operated by the technical staff of the

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laboratory. Calibration involved checking the '4C' Coulter reagent every third day, with day-to-day and within-day quality control effected by retesting six routine antenatal blood samples submitted the previous afternoon and stored at 4°C between analyses. The results and discussion that follow relate to the seven Coulter indices as follows: white cell count ($10^9/l$), WBC; red cell count ($10^{12}/l$), RBC; haemoglobin (g/dl), Hb; haematocrit, Hct; mean cell volume (fl), MCV; mean cell haemoglobin (pg), MCH; and mean cell haemoglobin concentration (g/dl), MCHC.

Results

The range of values obtained in the fresh samples is shown in table I together with the distribution in terms of normality accepted by this laboratory. The diversity of haematological abnormality in the patients studied is clearly reflected in these results.

A correlation matrix using all seven indices is shown in table II. If $r \pm 0.34$, the correlation between indices is highly significant ($p < 0.001$). The implications of a high degree of correlation between indices are considered in the discussion.

The effects of storage at room temperature may be assessed from changes in mean values and standard deviations of each index obtained with increasing dead time. These are detailed in table III. By Student's *t* test, there is no significant change in mean values for WBC, RBC, Hb, and MCH with increasing dead times compared with fresh samples. Although the change in RBC by 48 hours is statistically significant ($p < 0.001$) the difference in mean values is only $0.06 \times 10^{12}/l$ and obviously of no biological significance. On the other hand, changes in MCV and thus the computed indices, Hct and MCHC, attain statistical ($p < 0.01$) and biological significance by 24 hours, reflecting an approximate 3% swelling of the erythrocytes within this time.

Index	Normal Range	Range of Results	Distribution of Results		
			< Normal	Normal	> Normal
WBC	4.0-10.0	1.1-21.8	12	78	14
RBC	M 4.50-6.00	M 2.01-5.38	22	30	—
	F 4.00-5.00	F 2.19-5.96	18	31	3
Hb	M 13.5-18.0	M 5.9-16.3	23	29	—
	F 11.5-16.5	F 6.7-16.0	16	36	—
Hct	M 0.38-0.52	M 0.179-0.493	20	32	—
	F 0.36-0.50	F 0.225-0.484	21	31	—
MCV	83-101	60-108	23	74	7
MCH	27.5-34.0	16.7-35.5	27	70	7
MCHC	32.4-34.0	27.6-35.2	30	72	2

Table I The range of results in 104 patients and their distribution with respect to laboratory normals

	WBC	RBC	Hb	Hct	MCV	MCH	MCHC
WBC	—	0.22	0.19	0.20	-0.03	-0.06	0.09
RBC	0.22	—	0.80	0.84	-0.36	-0.31	0.06
Hb	0.19	0.80	—	0.97	0.22	0.30	0.44
Hct	0.20	0.84	0.97	—	0.16	0.19	0.27
MCV	-0.03	-0.36	0.22	0.16	—	0.96	0.40
MCH	-0.06	-0.31	0.30	0.19	0.96	—	0.63
MCHC	-0.09	0.06	0.44	0.27	0.40	0.63	—

Table II The correlation matrix of the Coulter S indices

Index	Dead Time				
	2 Hours	6 Hours	24 Hours	48 Hours	72 Hours
WBC	7.06 $\pm$ 3.45	7.10 $\pm$ 3.47	6.98 $\pm$ 3.40	7.10 $\pm$ 3.47	7.16 $\pm$ 3.49
RBC	4.249 $\pm$ 0.710	4.239 $\pm$ 0.714	4.269 $\pm$ 0.721	4.308 $\pm$ 0.728	4.309 $\pm$ 0.738
Hb	12.31 $\pm$ 2.14	12.26 $\pm$ 2.15	12.34 $\pm$ 2.12	12.38 $\pm$ 2.14	12.43 $\pm$ 2.15
Hct	0.3744 $\pm$ 0.0611	0.3744 $\pm$ 0.0607	0.3843 $\pm$ 0.0613	0.4035 $\pm$ 0.0671	0.4117 $\pm$ 0.0668
MCV	88.1 $\pm$ 8.2	88.3 $\pm$ 8.1	90.3 $\pm$ 8.2	93.7 $\pm$ 8.7	96.3 $\pm$ 9.5
MCH	29.22 $\pm$ 3.17	29.15 $\pm$ 3.24	29.26 $\pm$ 3.14	29.21 $\pm$ 3.17	29.37 $\pm$ 3.12
MCHC	32.84 $\pm$ 1.22	32.69 $\pm$ 1.53	32.13 $\pm$ 1.29	30.96 $\pm$ 1.28	30.33 $\pm$ 1.24

Table III The mean values with standard deviations for each index with increasing dead time

Whilst the actual changes in the mean values of WBC, RBC, Hb, and MCH are of no real significance, precision does deteriorate with time, particularly for the white cell count. This can be most readily appreciated by referring to the histograms (figs 1-4) which show the distribution of changes in WBC, RBC, Hb, and MCV at increasing dead times related to values in the fresh specimen.

The tolerable errors of Coulter counting in routine haematology have previously been based on replicate testing (Barnard, Carter, Crosland-Taylor, and Stewart, 1969; Pinkerton, Spence, Ogilvie, Ronald, Marchant, and Ray, 1970). In this study duplicate testing of samples with dead times of two and six hours has been adopted as such time delays commonly prevail in laboratory practice. The errors of reproducibility are expressed in table IV as standard deviations calculated from the formula $\pm \sqrt{\frac{d^2}{2n}}$

Index	Tolerated Error in Everyday Practice	Tolerated Error after 72 Hours' Storage
WBC	± 0.25	± 0.43
RBC	± 0.01	± 0.08
Hb	± 0.22	± 0.22
Hct	± 0.0098	—
MCV	± 1.1	—
MCH	± 0.53	± 0.46
MCHC	± 0.56	—

Table IV The tolerable error of reproducibility calculated by duplicate analysis

where d is the difference in index value between paired duplicates and n the number of pairs; also included for comparison are the tolerable errors (calculated from samples with dead times of two and 72 hours) of those indices relatively stable on storage.

Discussion

This study was purposely incorporated into the daily routine of the laboratory with no extra precautions taken to minimize the errors which arise in everyday practice. Nevertheless the standard deviations obtained by duplicate analysis of specimens with dead times of two and six hours are remarkably similar to those derived from replicate analysis of fewer specimens, uncontrolled for dead time and taken from a more normal population (Barnard *et al.*, 1970; Pinkerton *et al.*, 1970). Furthermore, although precision does tend to deteriorate with increasing dead time, the tolerated error in WBC, RBC, Hb, and MCH shows no significant change over 72 hours. Indeed, even after this period of storage, the tolerable error of the white cell count, the most variable parameter measured by the Coulter counter, is of the same order as with careful 'manual' enumeration of fresh blood by an experienced and meticulous observer (Berg, 1945).

Fig 1 Frequency distribution of differences in WBC at increasing dead times and the fresh sample value.

Fig 2 Frequency distribution of differences in RBC at increasing dead times and the fresh sample value.

Fig 3 Frequency distribution of differences in Hb at increasing dead times and the fresh sample value.

In practice this laboratory receives over 70% of its general practitioner samples within 24 hours of venepuncture and it is only after a weekend that any appreciable number (20%) have dead times greater than 48 hours. Thus the precision of screening of

postal samples by the Coulter counter is equal to, if not better than, manual analysis of fresh blood.

Turning to the interrelationships between Coulter counter indices of fresh blood, the high correlation ($r = 0.80$) between RBC and Hb is intuitively

Fig 4 Frequency distribution of differences in MCV at increasing dead times and the fresh sample value.

obvious: that it is not completely linear might be said to be the *raison d'être* of diagnostic morphological haematology. Furthermore a high degree of correspondence between Hb and Hct ($r = 0.97$) is not unexpected in view of experience with manual methods, but it is relevant to enquire whether both indices should be retained in everyday practice if the association is truly linear. As the Hb estimation is

precise and more stable, it should be retained. Of much greater interest is the high correlation between MCV and MCH ($r = 0.96$). Although a linear association has been propounded in recent correspondence (Rose, 1971), no derivation of the regression line was presented. Using the relevant values obtained with fresh blood, a regression line is derived and depicted in figure 5.

$$\text{Now familiarly, } \text{MCHC} = \frac{\text{Hb}}{\text{Hct}} = \frac{\text{Hb}}{\text{RBC} \times \text{MCV}} = \frac{\text{MCH}}{\text{MCV}}$$

Table V presents the MCHC derived from values of MCV and MCH interrelated by the regression line. It is pertinent to note that values for the normal ranges of MCV (83-101 fl) and MCH (27.3-33.9 pg) established by Okuno (1972) in healthy blood donors closely coincide with our regression line. This establishes the normal range for the Coulter MCHC

Fig 5 The regression line $\text{MCV} = 2.5 \text{ MCH} + 16$ with control lines placed at two standard errors of estimate.

MCV	MCH	MCHC
141	50.0	35.5
120	41.7	34.8
110	37.5	34.2
101	34.0	33.7
83	27.0	32.6
66	20.0	30.3
55	15.5	28.2

Table V The values for MCHC derived from values for MCV and MCH lying on the regression line $\text{MCV} = 2.5 \text{ MCH} + 16$

as 32.6-33.7 g/dl, and this is supported by our empirical finding that morphologically, red cell hypochromia is usually evident in samples where the MCHC < 32.4 g/dl. Consideration of the values presented in table V reveals the Coulter S MCHC to be more narrowly defined than the hitherto accepted range for the MCHC derived from manual methods.

The regression line relating MCV and MCH is in fact a simple function relating RBC, MCV, and Hb and the interrelationship of these indices in most blood samples will closely coincide with the derived line. There are, however, some obvious red cell abnormalities where the function does not hold, eg, microspherocytosis, where an MCHC > 36 g/dl is by no means unusual. It may prove possible to suspect the presence of other red cell changes associated with deviation in the usual relationships between red cell number, size, or haemoglobin content, eg, haemoglobinopathies, elliptocytosis, or mixed cell populations, by the recognition of a simple mismatch between measured values for MCV and MCH and those predicted by the regression line. Such an approach in blood screening programmes is technically feasible and considerably cheaper than the existing alternative, the development of machines that plot frequency distributions of red cell size (Brittin and Brecher, 1971). Nevertheless, in true diagnostic haematology there is still no real substitute for the microscopic examination of a well made blood film.

Although a narrow range for the Coulter MCHC has been described by other authors (Black, 1971; Brittin and Brecher, 1971; Okuno, 1972), all have concluded that its very narrowness limits its usefulness to an index of quality control, and that only if the MCHC is unusually high (> 36 g/dl) has it any diagnostic usefulness in that such samples require morphological scrutiny.

In contrast, we regard the MCHC as a much more

useful index for it is one measure of the interrelationships between red cell number, size, and haemoglobin content. Furthermore, we regard the narrowness of the range of the Coulter-derived MCHC as reflecting the increased precision of the Coulter S counter in the measurement of these basic red cell parameters. Thus an MCHC < 32.4 g/dl is a useful marker for hypochromia. A more sensitive measure of the normality between these parameters may, however, be the concurrence of MCV and MCH with the regression line $MCV = 2.5 \text{ MCH} + 16$. In the screening situation, consideration of the MCHC and both the MCV and MCH in terms of this function may improve the selection of samples requiring morphological scrutiny.

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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'. *J. clin. Path.*, 22, Suppl. (Coll. Path.), 3, 26-33.
- Berg, W. N. (1945). Blood cell counts their statistical interpretation. *Amer. Rev. Tuberc.*, 52, 179-220.
- Berkson, J., Magath, T. B., and Hurn, M. (1940). The error of the estimate of blood cell count as made with an hemocytometer. *Amer. J. Physiol.*, 128, 309-323.
- Black, P. J. (1971). Epitaph for the M.C.H.C. (Letter) *Brit. med. J.*, 2, 492-493.
- Brittin, G. M., and Brecher, G. (1971). Instrumentation and automation in clinical haematology. *Prog. Hemat.*, 7, 299-341.
- England, J. M., Walford, D. M., and Walters, D. A. W. (1972). Reassessment of the reliability of the haematocrit. *Brit. J. Haemat.*, 23, 247-256.
- Mattern, C. F. T., Brackett, F. S., and Olson, B. J. (1957). Determination of the number and size of particles by electrical gating: blood cells. *J. appl. Physiol.*, 10, 56-70.
- Okuno, T. (1972). Red cell size as measured by the Coulter model 'S'. *J. clin. Path.*, 25, 599-602.
- 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'. *J. clin. Path.*, 23, 68-76.
- Rose, M. S. (1971). Epitaph for the M.C.H.C. (Letter) *Brit. med. J.*, 2, 169.