Occasional articles

Current concerns in haematology 3: Blood count calibration

S M Lewis, J M England, R M Rowan

Introduction

A wide range of instruments is now available for blood counting; in general they are based on either aperture impedance (Coulter, Toa) or light scattering (Bayer Technicon). The parameters measured by these instruments include: red blood cell count (RBC); white cell count (WCC); platelet count (Plt); red blood cell sizing (MCV/Hct); haemoglobin (Hb); red cell parameters (MCH, MCHC).

Most semi-automated instruments are simply particle counters that are specifically adapted for haematological work. They will measure red blood cells, white cells or platelets when calibrated for the specified cell. Dilution is carried out independently, haemoglobin and packed cell volume (PCV) are measured on separate instruments and red cell parameters are calculated in the traditional way from the measurements of haemoglobin, red blood cells and PCV.

The more complex systems are multi-channel haematology analysers. They are fully automated with an internal dilution system and the basic units for counting are integrated with systems for cell sizing and measurement of haemoglobin. The most complex systems have automatic specimen handling and mixing, bar-coded identification, data storage facilities, quality control calculations and computer interfaces.

Cell counts on both semi-automated counters and multi-channel automated analysers are obtained by the number of pulses generated by cells in suspension, either as they pass through a small orifice located between two electrodes (aperture impedance), or as they pass in sheathed flow through a light beam (light scattering). As a first approximation it is assumed that the size of the pulse is proportional to the size of the cell, provided that the passage of the cell is not disturbed nor the cell distorted. The level of the pulse size which will be included in the count is controlled by a threshold, different settings of which will allow a count to be restricted to particles within specified size limits. It is thus possible, at least in principle, to provide an accurate count of specified cells without using a calibrator, provided the operator knows the size limits of the cells to be included and makes an appropriate correction for coincidence.

Many semi-automated counters that work by aperture impedance are designed to count

the number of cells present in a specified volume of suspension passing through the orifice—that is, they generate a count per unit volume from which the blood count can be derived after allowing for coincidence (by means of a chart or in-built algorithm) and the degree of dilution of the suspension.

By contrast, many multi-channel analysers do not count the number of cells in a given volume of suspension; instead they measure the rate at which cells pass through the sensing zone—that is, the counts per unit time, with automatic coincidence correction using an in-built algorithm. Because the coincidence corrected count per unit time obtained on the cell suspension cannot be directly related to the count per unit volume on the undiluted blood, the system has to be calibrated. The calibrator to be used must be diluted in the same diluent as the blood specimen and be compatible with the size and coincidence correction characteristics of the relevant blood cell type.

The ultimate goals are (1) to obtain accurate measurements of each blood count parameter and (2) to ensure harmonisation of results irrespective of which counter is used. These objectives are remarkably difficult to achieve for a number of reasons.

There has been confusion about the fact that most commercial materials are quality control preparations even when they are provided with label values and that there are very few calibrators available. As a rule the label values on control materials should be regarded as approximations, intended only as a guide when the preparation is used for checking precision—that is, the constancy of measurement values between batches of tests and day to day variations.

By contrast, the label values on a calibrant must be as accurate as possible. The problem is that reference methods have only been devised for use on fresh blood with a stability of a few hours; and these are so time consuming as to make it impractical in a routine laboratory.

Preserved blood—for example in acid-citrate dextrose or citrate-phosphate dextrose—is unsuitable as it behaves differently in each combination of automated counter and diluent. Stabilisation of cells—by partial or total fixation—affects their stability, inhibits osmotic shifts which occur when fresh blood is exposed to the diluent, and thus there are

NEQAS,
Royal Postgraduate
Medical School,
Du Cane Road,
London W12 0NN
S M Lewis
J M England
R M Rowan

Correspondence to:
S M Lewis

Accepted for publication
28 February 1991

differences in behaviour of fresh blood and stabilised cell suspensions, resulting in different MCV/Hct on different instruments.¹ A similar situation occurs with artificial materials such as latex particles. The discrepancies between these materials and fresh cells are reflected in what is described as the "shape factor". Shape factor is defined as the apparent volume of the cell divided by the true value.

In essence the instruments are designed to analyse fresh blood and not stabilised cell suspensions which may not undergo the same shape change when diluted in the manufacturer's specified diluent. Conversely, calibration by fresh blood is impractical except under special circumstances. A procedure which will provide the necessary link will therefore have to be designed.

1 Assignment of values to fresh blood

The ICSH has published a procedure for the assignment of values to fresh blood used for calibrating automated blood cell counters.² This requires direct measurements as follows:

- Haemoglobin by the ICSH cyanmethaemoglobin reference method.³
- Packed cell volume by capillary tube centrifugation.⁴
- Red cell count using a single channel semi-automated electronic counter² and correcting for coincidence.⁵
- Red cell indices (MCV, MCH, MCHC) by calculation.
- White cell count using a single channel semi-automated electronic counter.²
- Platelet count using a counter capable of measuring the ratio of platelets to red cells.⁶ The platelet count is then calculated from the ratio, the red cell count having been measured independently.

These procedures are time consuming and they require single channel electronic counters which may not be readily available in routine haematology laboratories. They can, however, be undertaken by reference centres.

2 Preserved blood

In blood stored at 4°C in acid-citrate dextrose (ACD) or citrate phosphate dextrose (CPD) red cell parameters are stable for about three weeks. This material is suitable for use in an external quality assessment scheme.

In the United Kingdom NEQAS, when CPD preparations have been used, participant results have shown excellent interlaboratory comparability for red cell parameters with each blood counting system, but there are inter-system differences.⁷

Commercial control preparations such as the Coulter 4C have been shown to have similar stability over 21 days with excellent inter-laboratory precision (CV of 0.61% for red blood cells, 0.52% for haemoglobin, 0.51% for PCV) when using class A glassware calibrated pipettes and the same type of counter in each laboratory.⁸

The British Committee for Standards in Haematology established a protocol for testing quality control material used with automatic blood counting apparatus.⁹

The objective of using this protocol is to ensure validity of the assigned values and to check continuing performance in routine use until the material reaches the end of its shelf life.

Direct measurements are made on fresh blood samples by reference methods as described above. Preserved blood preparations are then compared with the fresh blood on automated counters of various types in order to assign values to the preserved blood for each type of counter (fig 1).

The value to be assigned for each parameter is calculated from the formula:

$$d(p) = i(p) \times \frac{d(f)}{i(f)}$$

where d = direct value
i = indirect value
p = preserved blood
f = fresh blood

The stability of the preserved blood can be checked by assigning values at the beginning and end of the claimed stability period.

3 Artificial materials

Materials that have been suggested as surrogate blood cells fall into two main groups: artificial materials and natural blood cells that have been modified by fixative treatment. Artificial materials have included pollens, mould spores, yeasts and plastic polymers. None of these seems to have been suitable. This is partly because they do not have the relevant physical properties, but also, by their nature or method of preparation, they lack homogeneity. Mono-sized, spherical, polystyrene latex particles are now available in a series of defined sizes, with a CV of 2–3%. Apart from their homogeneity they have the major advantages that they are perfect spheres and they spontaneously form two-dimensional arrays with no gaps between the particles when a suspension is placed on a glass slide and covered by a coverglass. This allows their diameters to be measured directly using a calibrated stage micrometer, and their volumes to be calculated from:

$$V = \frac{\pi d^3}{6}$$

where V = volume
d = diameter

Two sets of latex particles have been estab-

Figure 1 Illustration of scheme for assigning values to preserved blood preparations.

Regression lines obtained when volume for latex particle, y , measured with "red cell calibrated" instrument is plotted against true volume, x , calculated from diameter

Instrument	Isoton	Orifice diameter $\times$ length (μm)	Regression line		
			a	b	
ZBi/C1000	laboratory 1	II	100 $\times$ 75	-7.787	1.623
	2			-2.605	1.485
S Plus IV	1	III	50 $\times$ 60	1.411	1.292
ZM/C256	1	III	50 $\times$ 100	-1.123	1.330
ZM/C256	1	III	100 $\times$ 75	-1.021	1.332

The regression line has an intercept, a , and a slope, b . Orifice diameters and lengths in the table are nominal values only in μm . The value of a is in fl and b is dimensionless. The value of b is the relative shape factor, latex:red cell ratio.

lished by the Community Bureau of Reference (BCR) of the Commission of the European Communities as Certified Reference Materials (CRM), with mean diameter (and volume) of 2.4 μm (5.7 fl) as a platelet equivalent, and 4.8 μm (58.7 fl) as a red cell equivalent, respectively.¹⁰ Other sizes have been prepared as working standards. These have mean diameter (volume) of 3.9 μm (31.3 fl) and 6.9 μm (176 fl), respectively. The last is a leucocyte equivalent.

Specifications of the CRMs include an approximate concentration. An accurate particle concentration has not yet been established for the BCR material, but preliminary studies indicate that there should be no major problem in establishing count standards in parallel to the size standards.

When measured on instruments calibrated with fresh blood cells the lattices give a different volume from that obtained by calculation from diameter (table). Though the different sized latex particles show linearity when their true volumes are plotted against instrument results. The regression lines have a non-zero intercept¹¹ (fig 2). But by recalibration of the counter (either by the laboratory or the service engineer), the non-zero intercept can be eliminated. The relation of true-volume:counter-volume ratio of the latex will then provide the relative shape factor, the latex:red cell ratio, for that particular instrument. The set of lattices would provide a means to check that the counter remains correctly calibrated and linear. In this way it would be a useful control, readily available, and easy to use in the individual routine laboratory on a regular basis, provided that the latex particles are injected directly into the tubing which would normally contain the diluted blood. Latex has the added advantage of remaining stable for several years.

When the relative shape factor has been

Figure 2 Linearity of different sized latex particles with different instruments.

established the counter can be used to obtain volume measurements of subsequent batches of latex particles. These measurements are thus directly traceable to the primary standard.

The followed protocol is designed to interrelate the use of the various materials in a calibration and control process to ensure harmonisation of results obtained with different counters and counting systems. It is hoped that reference laboratory authorities, manufacturers, and users will be encouraged to apply it.

A National Reference Laboratory

STEP 1

Assign values to fresh blood

Haemoglobin

RBC

PCV

MCV, MCH, MCHC

WBC

Platelet count

STEP 2

Assign values to preserved blood provided by manufacturers for validation as control material. Repeat tests to check validity until end of claimed shelf life.

STEP 3

Prepare latex particle batches at three levels of size. Assign values for size by direct measurement of diameter unless already certified. Certified reference materials (CRMs) are already available from the European Community Bureau of Reference (BCR).

B Manufacturers

B1 FOR MANUFACTURERS OF CALIBRATORS

STEP 1

Manufacture preserved blood calibrator.

Assign values applicable to counting systems.

STEP 2

Submit samples from each batch of calibrator to National Reference Laboratory for validation of assigned values.

B2 FOR MANUFACTURERS OF INSTRUMENTS

STEP 1

Assign values for size and concentration to batches of latex particles at three levels of size; to be certified as traceable to primary standard (BCR or National).

STEP 2

Check instruments before supplying to users to ensure that latex particle plots are linear and pass through zero.

Assign "shape factor" to each instrument individually.

C Users

At intervals and after major component change, check calibration and linearity of instrument by latex particles at three size levels. These would probably be commercially available from manufacturers.

- 1 England JM. Recommended methods for the assignment of assay values to stabilized suspensions. *Clin Lab Haematol* 1990;12(suppl 1):13–21.
- 2 International Committee for Standardization in Haematology. The assignment of values to fresh blood used for calibrating automated blood cell counters. *Clin Lab Haematol* 1988;10:203–12.
- 3 International Committee for Standardization in Haematology. Recommendation for reference method for haemoglobinometry in human blood and specification for international haemoglobinocyanide reference preparation Third edn. *Clin Lab Haematol* 1987;9:73–9.
- 4 International Committee for Standardization in Haematology. *Recommended methods for the determination of packed cell volume by centrifugation (WHO/LAB/89.1)*. Geneva: World Health Organization, 1989.
- 5 Lewis SM, England JM, Kubota F. Coincidence correction in red blood cell counting. *Phys Med Biol* 1989;34: 1239–46.
- 6 Lewis SM, Rowan RM, Kubota F. Evaluation of a prototype for a reference platelet counter. *J Clin Pathol* 1990;43: 932–6.
- 7 Wardle J, Ward PG, Lewis SM. Response of various blood counting systems to CPD-AI preserved whole blood. *Clin Lab Haematol* 1988;7:245–50.
- 8 England JM, Chetty MC, Garvey B, *et al.* Testing of calibration and quality control material used with automatic blood counting apparatus: application of the protocol described by the British Committee for Standardization in Haematology. *Clin Lab Haematol* 1983;5:83–92.
- 9 Crosland-Taylor PJ, Allen RWB, England JM, *et al.* Draft protocol for testing calibration and quality control material used with automatic blood-counting apparatus. *Clin Lab Haematol* 1979;1:61–4.
- 10 Thom R, Marchandise H, Colinet E. *The certification of monodisperse latex spheres in aqueous suspension with nominal diameter 2.0 µm, 4.8 µm and 9.6 µm. Community Bureau of Reference Report, EUR 9662*. Luxembourg: Commission of the European Communities, 1985.
- 11 England JM, Lewis SM, Rowan RM, Thom R. Surrogate materials for calibration and control: the use of latex particles as calibrants for red cell volume measurements. *Clin Lab Haematol* 1990;12(suppl 1):55–63.

Eponyms in pathology . . .

KULCHITSKY, Nicholas (1856–1925) was a Russian anatomist and histologist born in Kronstadt. In 1893 he became professor of histology at the university of Kharkov and was later appointed director of education in Kasan. In 1897 he described the endocrine cells of the small intestine which now bear his name. After the Russian revolution of 1917 he came to England and worked at University College, London. He died there on January 30 1925 as the result of an accident.