Current concerns in haematology 3: Blood count calibration

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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 simple 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
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.  

Commercial control preparations such as the Coulter 4C have been shown to have similar stability over 21 days with excellent interlaboratory 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.  

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

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  
- $i = \text{indirect value}$  
- $p = \text{preserved blood}$  
- $f = \text{fresh blood}$  

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. Monosized, 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:  

$$V = \frac{4}{3}\pi d^3$$  

where  

- $V =$ volume  
- $d =$ diameter  

Two sets of latex particles have been estab-
Regression lines obtained when volume for latex particle, \( y \), measured with "red cell calibrated" instrument is plotted against true volume, \( x \), calculated from diameter

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Isotom</th>
<th>Orifice diameter ( (\mu m) ) \times \text{length}</th>
<th>Regression line ( a )</th>
<th>Regression line ( b )</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZBi/C1000</td>
<td>laboratory 1 II</td>
<td>100 ( \times ) 75</td>
<td>(-7.787)</td>
<td>(1.623)</td>
</tr>
<tr>
<td>S Plus IV</td>
<td>2</td>
<td>50 ( \times ) 60</td>
<td>(-2.405)</td>
<td>(1.485)</td>
</tr>
<tr>
<td>ZM/C256</td>
<td>1</td>
<td>50 ( \times ) 100</td>
<td>(-1.123)</td>
<td>(1.330)</td>
</tr>
<tr>
<td>ZM/C256</td>
<td>1</td>
<td>100 ( \times ) 75</td>
<td>(-1.021)</td>
<td>(1.352)</td>
</tr>
</tbody>
</table>

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

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.
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.