Quality Control in Blood Cell Analysis

P. W. Helleman
Laan van de Eekharst 76, 7823 AS Emmen, The Netherlands

Abstract. In the literature various methods are described for quality control in the determination of haemometric and haemocytometric values. In most clinical laboratories, commercial blood cell control material is utilised in internal quality control. Although it is claimed that the materials do not change markedly within 4 to 6 weeks, variations may occur. The values assigned to the materials are determined by utilising various blood cell analysers in selected laboratories, and may vary according to the type of instrument. Since the analytical results should be the same for every type of instrument, one may deduce that, by using such materials, only the repeatability and reproducibility, i.e., the constancy of performance of the instrument is checked. Although the use of the materials has some advantages, it is clear that accuracy is not guaranteed where there is lack of a calibrator.

In the National Institute of Public Health and Environmental Hygiene (RIVM) in the Netherlands, efforts were made to improve on the methods of quality control in haematology. After studying five methods mentioned in the literature for the preparation of stabilised erythrocytes, the method described by Benedek (1966) was found to be the best when taking into consideration the shapes of the cells, the suitability for counting, and the stability. In a contract with the Council of Europe the method was further developed.

Thorough studies were also undertaken into the performance of cell counting instruments. In collaboration with nine clinical laboratories we succeeded, taking into account the basic anomalies of the various types of blood cell counting instruments, in determining the concentration of a suspension of stabilised cells (Stabicells) to a tolerance of less than 2%. As its lifetime is virtually unlimited, this suspension may be considered to be a calibrator.

A comparable procedure was used to prepare a calibrator for the automated determination of haemoglobin concentration. The concentration could be shown not to deviate markedly within a period of 3 years.

Using a combination of technical anomaly corrections with quality control by daily calibration, we set up a system independent of external control. Since in animal haematology, problems are greater than in human haematology, and external support is less available, application of the system is strongly recommended in animal haematology.

Keywords: Quality control; Blood cell analysis; Red cell concentration; Haemoglobin concentration; Haematocrit value; Red cell characteristics; White cell concentration; Thrombocyte concentration

Determination of the MCH

In automated haematological analysis the mean erythrocyte haemoglobin mass (MCH) is the only erythrocytometric value which can be determined accurately. Determination of the other erythrocyte characteristics, such as mean erythrocyte volume (MCV) and mean erythrocyte haemoglobin concentration (MCHC), is far less accurate, since until recently the determination of the MCV in certain types of instruments, and the determination of the haematocrit value (Ht) in others, have been prone to a number of errors.

With the exception of the Techicon H-1, modern blood cell analysing instruments can only determine the MCH through the separate determination of the haemoglobin concentration (Hb) and the erythrocyte count.
(RBC). The implications of determining the MCH with the Technicon H-1 will be discussed later.

The Determination of the RBC

Notwithstanding the high precision of erythrocyte counts using blood cell counting instruments, in practice it is difficult accurately to determine the erythrocyte count in the original blood sample. This is because it is nearly impossible to eliminate all sources of error, or correct for variations caused by aberrations of the instruments. If a variation is proportionally related to the RBC, a calibrator may be used to correct for the variation. Until recently, calibrators were not available for this purpose, and it was therefore not possible to be certain of the accuracy of the counts. Because of this, in most instances control preparations were used as a substitute, using values determined by the supplier. Since these preparations have limited shelf life, the assigned values may change with time, moreover, the preparations may have differing values depending on the instruments used, and therefore suffer the errors typically associated with the various types of instruments.

Because of the lack of a calibrator in cell counting, in the framework of a contract between the National Institute of Public Health and Environmental Hygiene (RIVM) in the Netherlands and the Council of Europe, an attempt was made to develop a true calibrator. It became clear that it was not possible to prepare a suspension of native erythrocytes with a haematocrit value, MCV and RBC count stable for a period longer than 14–16 weeks. To overcome this difficulty, the philosophy that a calibrator should have the characteristics of native erythrocytes, was rejected. Because natural particles like yeast and pollens, or artificial particles produced from latex or polyethylene, appeared to aggregate rather quickly, and because mono-sized particles prevent accurate correction of the coincident loss and accurate adjustment of the lower threshold setting, efforts were focused on particles which produced signals identical to native erythrocytes.

After comparing the suitability of erythrocytes stabilised by various types of chemicals (such as glutaraldehyde, formaldehyde, osmium tetroxide and sodium sulphate), encouraging results were obtained with material fixed with formaldehyde (Benedek 1966; Mortensen and Howell 1966) or glutaraldehyde (Lewis and Burgess 1966; Archer 1970). During further studies it became apparent that formaldehyde fixation was preferable to glutaraldehyde since aggregation of the cells was less (coefficient of variation (CV) 2%, compared with 5% in the case of glutaraldehyde fixed erythrocytes).

By applying slight modifications, cells were obtained having a shape similar to fresh erythrocytes, which was retained over a long period of time and was therefore suitable as a calibrator in erythrocyte counting. As a valid concentration value may only be assigned if a reference counting method is used with various types of instruments in a number of laboratories. Co-operation was sought with other laboratories. Five other laboratories were willing to study the peculiarities of their counting instruments, to train their personnel on a voluntary basis, and to perform internal quality control experiments.

For reasons which will become clear, only so-called absolute counters should be used for the reference method. As a consequence, counting instruments which are not able to deliver a constant and accurate sample volume to the detector cannot be used. Before a reference method is introduced, the various parts of the instrument used and the counting procedure should be studied (Helleman 1978a). An overview of the most important aspects is given in the following paragraphs.

Since it is impossible to correct for count loss due to coincidence in undiluted blood, the blood must be diluted substantially. The dilution factor (Fd) must be accurately known, as well as the count-volume (Vc), which is the volume of diluted blood used for counting.

After amplification the resulting signals generated by the cells passing through the detector, must be counted as accurately as possible. Because of coincidence of particles in the detector, and changes of the signals in the amplifiers and in the counting device, only a part of the total signals will be counted (Helleman 1970, 1972). In some counting instruments, the number of signals is reduced prior to their arrival at the counting device. The subunit (called the count-divider) responsible for this reduction employs a reduction-factor (Fe) which in most instances is constant, with values such as 24, 50 or 100. Dependent on the reduction-factor, the dilution factor and the count volume, a decimal point is inserted somewhere between the digits offering a value close to the actual concentration. If other sources of error are excluded, the departure from the original sample count is caused by count loss due to coincidence. This count loss is related to the number of cells counted (Nc). When not considering count loss due to coincidence, the relationship between the RBC and registered value (Nr) may be expressed by:

$$\text{RBC} = 10^n \cdot N_r F_c F_d V_c$$

where n is the number of digits to the right of the decimal point, while Nc, Fe equals Nc. The value of Nc is not only important to establish the magnitude of the coincidence loss, but also to establish the theoretical measure of precision. This latter value is important in deciding whether the magnitude of the variation caused by random errors, is acceptable.

The dilution procedure may be most efficiently checked by using calibrated pipettes with large volumes (0.5–1 ml) and calibrated volumetric flasks with volumes in accordance with the required dilution factors (e.g. 500 and 200 ml). As it is essential to eliminate effects of viscosity of the sample, pipettes must be calibrated as ‘to contain’ pipettes. This is best done