Control of capacity

The technique for measuring the capacity of a Ni–Cd battery is complicated. However, the following methods have been found to be practical.

- Voltage measurement with a multimeter. However, the results are difficult to interpret because the difference between complete charge and total discharge is only 0.24 V.
- Use of a battery test system.
- Voltage measurement prior to and after a load of 10 times the stated recharging current of the battery. A fully charged new battery will show only a minor difference, while an old battery will give a reading of not more than 1.0 volt after the load.

Notes on batteries

Before buying, or accepting as a gift, a piece of equipment that uses batteries, check that replacements are readily available. Also, remember that batteries are expensive to buy and can be difficult to store satisfactorily. Sometimes, mains or low-voltage powered equipment might be a better option.

When the equipment is not in use remove the batteries, to avoid possible corrosion and damage to the instrument.

Cell counters

Cell counters are used for haematological measurement. Semi-automated and automated cell counting has proved to be much more reliable than microscopic cell counting, because a far greater number of cells can be counted rapidly in a specimen by an analyser system, resulting in greater precision. However, the improvement in precision does not necessarily imply a simultaneous improvement in accuracy. For the study of pathological cell types, microscopic examination of a blood smear by an experienced investigator is still most valuable, and in many instances the method of choice.

A number of different principles are used in cell analysers:

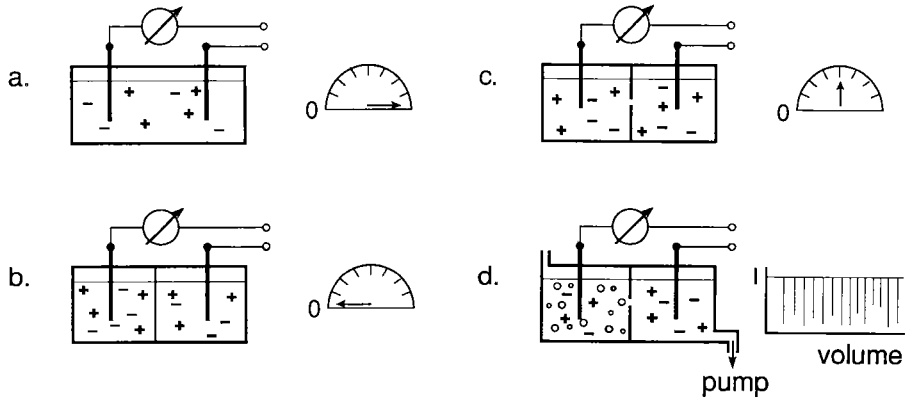
– impedance measurement,
– light scattering,
– centrifugation and quantitative buffy-coat analysis.

Using these techniques, all major classes of blood cells (erythrocytes, platelets, leukocytes) can be identified and even subclasses (granulocytes and lymphocytes) can be measured. More sophisticated instruments, which combine measurement of cell size and cell fluorescence, or cell size determination and immunofluorescence, allow subclassification.

Impedance measurement is most commonly used for cell counting. This principle of measurement takes advantage of the fact that blood cells are less conductive than the diluent electrolyte. The principle of the method is explained below.

1. When an electrical potential is applied to two electrodes dipped into an electrolyte solution, an electric current can be measured owing to the transport of ions from one electrode to the other. The magnitude of the current will depend on the concentration of the ions in solution. It will be constant if the transport of ions is constant (Fig. 2.12a).
2. If the electrodes are separated by an insulator, the flow of electric current will drop to zero (Fig. 2.12b).

Fig. 2.12. Measurement of impedance as a method of counting cells.



WHO 93107

3. The current will reappear if a small aperture is introduced into the insulator, but the magnitude of the current will be small, because the insulation is still partially effective (Fig. 2.12c).
4. A small particle such as a blood cell with a conductivity lower than that of the electrolyte solution, passing through the aperture from one chamber to another, will temporarily decrease the current because a smaller volume of the electrolyte solution is able to pass through the aperture at the same time. The current will regain its original value when the particle has passed through the aperture (Fig. 2.12d).

When a cell-containing fluid is sucked through the aperture of an insulator separating two electrode chambers, each change in current (registered as a pulse) indicates the passage of a particle through the aperture, thus allowing the cells to be counted. Furthermore, the magnitude of each pulse is proportional to the size of the particle. Simple cell counters register only the number of pulses above a certain threshold. More sophisticated counters also register the magnitude of each pulse and show the distribution of the pulse magnitudes, thus indicating the distribution of particle sizes in the population of cells. The concentration of cells in the sample is measured by counting the number of pulses for a known volume of fluid.

The principle of measurement implies that each measured pulse is attributed to the passage of a single particle through the aperture of the separating membrane. Therefore, a blood specimen having a cell concentration of about 5 million cells per microlitre must be appropriately diluted (in general, 200- or 250-fold) to ensure that individual cells are counted. Sometimes, errors resulting from the passage of more than one cell still occur in specimens with high cell concentrations. These errors can be avoided by:

- Further dilution of the blood specimen and a repeat measurement.
- Application of a correction factor to the number of cells counted to allow for coincidence. This factor varies with the cell concentration and may be taken from a table provided for the instrument by the manufacturer. Modern instruments have a built-in calculation programme to allow for coincidence.

Most cell counters cannot be calibrated by the user. Therefore, a standard prepared, or purchased, cell suspension can serve only for control of precision but not for accuracy. The given cell concentration in commercially available control materials can be used only for measurement with a specified instrument; different results will be obtained with instruments with a different threshold limit.

The determination of cell size by impedance measurement is influenced by a number of technical factors, which may vary from one instrument to another, and also by the type and shape of the particles being measured. For example, the magnitude of the signal from a discoid cell, e.g., an erythrocyte, is different from that from a spherical cell, e.g., a leukocyte, even if the cells have the same volume.

Cell counters using the impedance principle count the combined total of red blood cells which have a cell volume between 70 and 120 fl (femtolitres), and leukocytes, which have a cell volume between 100 and 350 fl. The results are given in terms of red blood cell count, ignoring the negligible proportion of leukocytes (usually only 0.2%—10 000 leukocytes compared with 5 million red cells per ml). However, a considerable error may occur in specimens with a much higher proportion of leukocytes, e.g. those from patients with pronounced anaemia and high leukocyte concentrations.

For leukocyte counting, erythrocytes are lysed by an ionic or non-ionic detergent in solution—a procedure that does not lyse leukocytes within a certain time. However, the size of the leukocytes, and particularly of the polymorphonuclear cells, may vary. Erroneously low counts may be obtained at high leukocyte concentrations, when for example two cells pass through the aperture simultaneously, while being registered only as a single pulse. Such errors can be eliminated by greater dilution of the specimen, as mentioned above.

Measurement of platelet concentration is more prone to error, because of their small cell size (1–10 fl). It is particularly important that the buffer solution used for the dilution of the blood specimen is free of dust particles; these solutions must be filtered through a filter of 1 μm pore size. Absence of dust is also important for counting of red and white blood cells, although the presence of impurities with a particle size of less than 1 μm is of less importance.

General errors that will affect measurements of cell concentrations are:

- unsatisfactory blood sampling and storage;
- inadequate dilution of the specimen;
- fibrin precipitates or cryoprecipitates in the specimen;
- inadequate lysis of red blood cells when counting white blood cells;
- lack of homogeneity in the distribution of blood cells in the dilution.

Technical errors in cell counting may result from:

- fluctuations in the electric current;
- incorrect setting of the size threshold of the instrument;
- dust particles in the diluent;
- leakage in the suction system of the instrument;
- partial or total obstruction of the aperture;
- multiple cell passage at high cell counts;
- carry-over from one measurement to a subsequent measurement.

To avoid these errors, the following measures must be routinely undertaken:

- the cell counter should be connected to an electrical stabilizer;
- the aperture between the electrolyte chambers should be checked after each series of measurements, and cleaned with a small soft brush, if necessary;
- the electrodes must be checked, to ensure that they dip into the electrolyte solution in both chambers;
- the fittings of removable parts should be coated with silicone grease to avoid air-leakage in the suction system;
- the bottle of diluent solution should be checked daily, and the waste solution discarded;
- the suction system should be cleaned with diluent solution to avoid carry-over effects;

- at the end of each working day, the suction system should be cleaned with detergent solution and afterwards with the diluent solution;
- the aperture of the glass cuvette must always be kept immersed in diluent solution to avoid obstruction.

Further maintenance procedures (e.g., cleaning of mercury, etc.), must be carried out according to the manufacturer's instructions.

Centrifuges

Basic principles

A centrifuge is a machine that applies a sustained centrifugal force (i.e., a force due to rotation) to impel matter outwards from the centre of rotation. This principle is used to separate out particles in a liquid medium by sedimentation. The physical basis of the separation is the action of a centrifugal force on the rotating particles, which increases with the radius of the rotational field and the velocity of the rotation. The rate of sedimentation is determined by the density of the particles. Dense particles sediment first, followed by lighter particles. Depending on the conditions, very light particles may even remain in suspension.

The relative centrifugal force is related to the number of revolutions of the rotor per minute according to the formula:

$$\mathrm{RCF} = 1.118 \times 10^{-6} \times r \times n^2$$

where RCF = relative centrifugal force (g)

r = radius in millimetres from the centrifuge spindle to point of tube,

and

n = no. of revolutions per minute.

The relative centrifugal force can easily be calculated from a nomogram (Fig. 2.13), where the radius is measured from the centre of the rotor to the middle of the tube placed in the radially oriented rotor bucket; e.g., if the radius is 75 mm, the speed of rotation must be 2500 revolutions per minute to develop a centrifugal force of 520 g. It is important that the temperature in the centrifuge does not exceed 37 °C, otherwise degradation of some constituents of the specimen may occur.

There are two main types of centrifuge: preparative and analytical.

Preparative centrifuges are used to separate the solids suspended in biological samples from the supporting fluid. This is the most common type of centrifuge, and they are fitted with swing-out, or fixed-angle, heads.

Preparative centrifuges vary in their sample capacity and size, from floor-standing to small capacity centrifuges that can be sited on a bench. Some are fitted with internal wind shields to protect the operator from contamination by any aerosols that may be formed. This is now a mandatory safety requirement in many countries.

Two types of preparative centrifuge are currently used—mechanical and electrical —although the majority are electrical centrifuges.

Analytical centrifuges may be used to quantify one or more solid components in a mixed suspension. The only centrifuge of this type used in medical laboratories is the microhaematocrit centrifuge.