



RECOMMENDED METHODS FOR THE DETERMINATION OF PACKED CELL
 VOLUME BY CENTRIFUGATION

Prepared on behalf of the World Health Organization

by

The Expert Panel on Cytometry¹ of the International
 Committee for Standardization in Haematology

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1. INTRODUCTION

Determination of the packed (red) cell volume (PCV) is intended to measure the relative volume occupied by the red cells (erythrocytes) in capillary or venous samples of whole blood. It is measured by means of centrifugation and is expressed as a decimal fraction. The terms haematocrit-value (haematocrit ratio), red cell volume fraction and packed cell volume are considered synonymous.

The determination of PCV is a simple and reliable method for detecting the presence or absence of anaemia or polycythaemia. Indeed the PCV measured by centrifugation is more reliable for monitoring patients with polycythaemia than is the PCV calculated on many automated cell counters.

The PCV may be used, in conjunction with the red cell count or haemoglobin concentration, for calculating the mean cell volume or mean corpuscular haemoglobin concentration, respectively:

$$\text{MCV (fl)} = \frac{\text{PCV (l/l)} \times 10^{15}}{\text{number of red cells per litre}}$$

$$\text{MCHC (g/l)} = \frac{\text{haemoglobin concentration (g/l)}}{\text{PCV (l/l)}}$$

The recommended working methods which are described in this document have been selected on the basis of their widespread use and acceptable levels of accuracy and precision using relatively simple equipment. Two methods are available, the micro-method and the macro-method. In both methods a column of blood is centrifuged in a tube of uniform bore, which is closed at one end. Centrifugation is performed until the "packing" of cells is as complete as possible, i.e. until further centrifugation, under the same conditions, leaves the cell column unaltered in length. For the micro-method, this is at least 5 minutes in a special high-speed centrifuge. The length of the red cell column after centrifugation relative to the total length of the column gives the PCV.

However, with both the micro-method and the macro-method plasma remains trapped between the centrifuged red cells and increases the apparent length of the red cell column by about 2% in normal blood and even more in certain abnormal conditions, notably iron deficiency, thalassaemia, spherocytosis and sickle cell disease. This must be taken into account when a high degree of accuracy is required. For this purpose a reference method has been developed with a correction for trapped plasma¹. The reference method is too time-consuming to be used in routine clinical practice.

2. BLOOD SAMPLES

Either venous or capillary blood can be used for the micro-method but venous blood is required for the macro-method.

A venous blood sample should be taken under minimal stasis, i.e. with just sufficient compression to ensure a free flow of blood, and mixed with an anticoagulant that will neither shrink nor swell the cells nor significantly dilute the blood. For example, 1.2 to 1.5 mg dipotassium EDTA/ml of blood is suitable for venous samples. Containers may be prepared or purchased containing anticoagulant. A label for the patient's identity and marked to indicate the correct quantity of blood to be added in relation to the amount of the anticoagulant should be affixed. It is advisable that the container should be large enough so that there is an equal amount of air present when the correct amount of blood is added. This enables proper mixing and allows oxygenation of

¹ ICSH recommendation for reference method for determination by centrifugation of packed cell volume of blood. J. Clin. Path. 1980; 33: 1-2.

the blood during the process. The PCV of oxygenated blood is about 1% less than that of deoxygenated blood, due to the loss of carbon dioxide on aeration.

If capillary blood is used, special microhaematocrit tubes, which require not more than 50 μ l of blood, may be filled directly from a puncture site on the finger, ear or heel. The internal surface of these tubes must be coated with anticoagulant. An amount of 2 IU of heparin per tube is suitable. A free flow of blood without tissue fluid is essential.

In many samples an increase of the PCV is found on storage at 20°C and it is thus recommended that centrifugation be performed within 6 hours of collecting the blood sample.

3. THE MICRO-METHOD

3.1 Materials and apparatus

- (a) About 50 μ l of blood is required for each determination.
- (b) The special disposable glass capillary tubes are 7.5 cm long with a uniform bore of approximately 1 mm. They are made of glass with a wall thickness of 0.2-0.25 mm. In some countries, standard-making authorities have established control procedures to ensure that tubes used for determining the PCV conform to these specifications. The tubes are not graduated. It is recommended that one end should have an identifiable marking, e.g. a red band, if it contains a dried film of anticoagulant (2 IU heparin per tube), so that it may be filled directly with capillary blood.
- (c) A special sealing compound is required for closing one end of the tube after filling.
- (d) The centrifuge, specially designed to hold a number of tubes (e.g. 24) in numbered positions, should have a radius greater than 8 cm and be capable of sustaining a constant force corresponding to 10 000-15 000 g at its periphery for at least five minutes. The temperature should not rise above 45°C in this time. If necessary, the test should be carried out in a special cool room or in a laboratory provided with air-conditioning. There should be sufficient time between runs to allow the centrifuge to cool down.

3.2 Method

- (a) The tubes are filled with blood for two-thirds to three-quarters of their total length and the distal dry, unfilled end sealed by forcing a special sealing compound into it. In all cases the seal must provide a flat internal bottom.
- (b) After the filled tubes are placed in the centrifuge and the position of each tube is recorded, they are spun at 10 000-15 000 g for five minutes. In some cases (see 7.4) packing will not be complete in five minutes. In such cases the gravitational force applied to the top of the red cell column is less than usual, and the tubes should be spun for a further three minutes.
- (c) The tubes are read one at a time. The ratio of the length of the red cell column to the total length of the column of blood can be calculated from measurements obtained by placing the tube against arithmetic graph paper or against a ruler. The platelet and leukocyte layers, which form the buffy coat, are excluded as far as possible. Special reading devices are also available from some centrifuge manufacturers. If the tubes are not read immediately they are removed from the centrifuge and placed in a vertical position until they are read. They are then discarded into disinfectant solution.

- (d) When the test is done in duplicate for the purpose of quality control, the two results should not differ by more than 0.02 l/l.

4. MACRO-METHOD

4.1 Materials and apparatus

- (a) About 0.6 ml of blood is required for each determination.
- (b) A Wintrobe haematocrit tube is used. It is a thick-walled graduated tube of uniform bore with a flat seal at the base and is graduated for 100 mm from its base. The internal diameter should be in the range of 2.5-3.0 mm. A laboratory centrifuge is required. The arm length should be 15 cm or more as measured from the axis of rotation to the base of the buckets that hold the tubes. The centrifuge should be capable of sustaining a force corresponding to about 2300 g at the base of the buckets [see 4.2 (b)]. The temperature of the samples should not rise above 45°C during centrifugation.

4.2 Method

- (a) A Wintrobe tube is filled from the bottom upwards with well mixed anticoagulated blood by means of a Pasteur pipette or a syringe equipped with a long needle. Care must be taken to exclude air bubbles. After the pipette or needle has been removed, the column of blood should be exactly at the 100 mm mark. Because slight evaporation may occur during centrifugation it is not possible to estimate the original length of the column reliably when the PCV is being read after centrifugation.
- (b) The tubes are centrifuged for 30 minutes at 2000-2300 g (see Table 1). For most purposes this time is adequate and practical. In some cases (see 7.4) the packing will not be complete after this period and the centrifugation times should be extended to 60 minutes.

Table 1: Revolutions per minute required for approximately 2000-2300 g at various distances from the axis

Radius	Revolutions per minute for	
	2000 g	2300 g
15 cm	3400	3600
20 cm	2900	3100
25 cm	2600	2800

$$N = \sqrt{\frac{G}{0,0000118 \times R}} = 291 \sqrt{\frac{G}{R}}$$

N - revolutions per minute
 G - centrifugal force in g
 R - distance in cm from axis

- (c) The tubes are removed from the centrifuge and the height of the red cell column, expressed as a fraction of the original length of the column of blood (100 mm), is read to give the PCV. The platelet and leucocyte layers, which form the buffy coat, are excluded as far as possible.

- (d) The tubes should be inverted and placed in water immediately after use. Each tube is cleaned by forcing water down to the bottom displacing the column of red cells. A long steel needle inserted to the bottom of the tube is convenient for this purpose. It is then rinsed with distilled or deionised water and dried in warm air.

After some use the tubes tend to accumulate a film of proteins and carbonate on their internal surface. They should therefore be soaked once a week in dilute hydrochloric acid (0.1N - 0.1 mol/l) for 24 hours, then cleaned and dried as before.

5. RISK OF INFECTION

At all times blood samples must be handled with care to avoid contamination to the operator and to the working area. With high-risk materials the operator should wear protective gloves and if possible carry out the procedure in a safety cabinet.

If breakage is suspected whilst the centrifuge is running, the motor must be switched off and the equipment remain closed for 30 minutes. If breakage is discovered only on opening the centrifuge, the lid should be replaced immediately and left for 30 minutes.

Protective gloves must be worn for cleaning the centrifuge. Forceps or cotton wool held in forceps should be used to pick up glass debris. All broken tubes, glass fragments, buckets, trunnions and the rotor must be placed overnight in disinfectant. The centrifuge bowl must be swabbed with disinfectant, left for 30 minutes, reswabbed, washed with water and dried. Swabs must be treated as infected waste and disposed of accordingly.

Activated glutaraldehyde (2% solution) is suitable for viral decontamination. Hypochlorites must not be used on centrifuges since they are corrosive.

6. EXPRESSION OF RESULTS

In conformity with the ICSH/IFCC/WASP recommendation for the use of SI units in clinical laboratory measurement, the PCV should be expressed as a fraction, e.g. 0.45 l/l, and not as a percentage.

6.1 Reference values

There are no reference values available for all populations; different values are given in various text books. The following are commonly accepted values for adults between 20 and 50 years of age living at sea level.

Males:	0.47 l/l (95% limits: \pm 0.07)
Females:	0.42 l/l (95% limits: \pm 0.05)

The PCV may also fluctuate with exercise and with posture.

7. SOURCES OF ERROR

7.1 Sampling errors

The blood sample may be haemoconcentrated due to the prolonged use of a tourniquet, or it may be diluted with interstitial fluid, especially where there has been difficulty in venepuncture or failure to obtain free flow of capillary blood. It may be partially haemolysed by being drawn or expelled forcibly through a fine bore needle. Clots may be formed if interstitial fluid has been mixed with the blood after a difficult venepuncture, or if the blood is not immediately mixed with anticoagulant.

Some samples of blood taken in a syringe tend to sediment significantly in less than one minute. Aliquots taken from the same syringe may then have different proportions of cells if the blood in the syringe is not mixed before the PCV tube is filled.

7.2 Tube and filling errors

Tubes which are not clean and dry cause false results. Poor quality tubes may not be of uniform bore or the seal at the bottom may not be flat. If filling of the macrohaematocrit tubes is not precisely to the 100 mm mark, the reading should be noted before centrifugation and used for calculating the result [see 4.1 (c)]. Presence of air bubbles in the column when the tube is being filled makes correct reading impossible.

7.3 Reading errors

The white cells and platelets (buffy coat) must be excluded from the reading for the height of the red cell column, but this may be difficult when the layers are poorly defined. Reading errors due to parallax must be avoided.

7.4 Packing errors

The red cell column will always include some trapped white cells and platelets and, particularly, trapped plasma. The latter will be increased in patients with polycythaemia and also, sometimes considerably, in certain other conditions, such as iron deficiency anaemia or sickle cell disease. An increase is also found when centrifuges with short arms are used or if the samples are centrifuged at low rather than high centrifugal force. The adequacy of the centrifuge and of the spinning time may be checked by centrifuging a normal and a polycythaemic blood sample in duplicate for 5, 7, 9 and 11 minutes successively in a microhaematocrit centrifuge and for 30, 45 and 60 minutes successively in the macro-method. The shortest time after which no further decrease in the length of the red cell column occurs is considered the adequate spinning time for these types of specimens.

It is advisable, when quoting PCV in publications, to give details of the tubes used, the gravitational force and the time of centrifugation.

8. COMPARISON OF THE TWO METHODS

In routine practice the micro-method is recommended. In most countries it is replacing the macro-method, as the advantages of the shorter time for centrifuging, the small quantity of blood required and the smaller amount of trapped plasma outweigh the greater difficulty in obtaining an accurate reading. On the average, the PCV by micro-method is lower than that measured by the macro-method by approximately 0.01-0.02 l/l.

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