Laboratory diagnosis and investigation of anaemia

**AUTHOR** Ken Campbell, FIBMS, CertHMS, is clinical information officer, Leukaemia Research Fund.


Anaemia is one of the most common conditions encountered in clinical practice. This article outlines the methods used to investigate anaemia and discusses the significance of laboratory diagnosis in treating this condition.

Laboratory investigations play an essential part in diagnosing anaemia, establishing its aetiology and determining and monitoring its appropriate treatment. Nurses working in primary care settings and on the wards need knowledge of the relevant laboratory values as effective as nurses specialising in haematology.

**Anaemia**

Anaemia is one of the most common clinical conditions encountered. It can vary from a mild, clinically quiescent condition to a serious, incapacitating disability. Its causes vary from a modest mismatch between the iron requirements and intakes of a pregnant woman to the only overt sign of advanced malignant or other systemic disease.

Treatment may be simply an oral course of haematinics – the substances required for maintenance of a normal blood count such as iron, vitamin B<sub>12</sub> and folic acid. It may require life-long parenteral administration of vitamin B<sub>12</sub> or it may consist of effective treatment of the underlying aetiology. Strictly speaking, anaemia is not a disease per se. It is a clinical feature of other conditions such as haemorrhage, chronic renal disease or infection, liver disease or dietary deficiency.

A pseudo-anaemia can occur during pregnancy when expansion of plasma volume occurs without a corresponding increase in red cell numbers. This is not a true anaemia as the tables of normal ranges used to define anaemia stipulate distinct values for pregnant women.

One feature common to all forms of anaemia is the central role played by laboratory investigations in diagnosis, in establishing aetiology and in determining and monitoring appropriate therapy.

**Sample collection**

**Protocol**

As with all laboratory testing the single most important requirement for an anaemia test is that the laboratory be provided with a properly labelled sample, taken appropriately, put into the correct preservative, and stored and transported in a suitable manner. If any of these criteria are not satisfied it may mean that the test cannot be performed or that the results are invalidated.

Containers must always be labelled immediately after being filled, that is before leaving the bedside. Any well-run laboratory will destroy all unlabelled samples on receipt. A detailed summary of recommendations for sample labelling procedure can be found in the transfusion guidelines of the British Committee for Standards in Haematology at www.bcshguidelines.com/pdf/tme203.pdf

Any nurse regularly collecting samples or assisting at collection will quickly become familiar with the appropriate preservatives for different tests. It is very important to be aware that the colour coding of bottles (to indicate which test they are suitable for) is not standard.

For this reason, it is vital that all nurses who have changed working locations should familiarise themselves with the colour codes used at the new location. All laboratories produce handbooks that list local requirements for different tests. These should also contain a listing of sample bottle colours and markings.

**Learning objectives**

Each week *Nursing Times* publishes a guided learning article with reflection points to help you with your CPD. After reading the article you should be able to:

- Identify how sample collection contributes to result accuracy;
- Know the different investigations that lead to the diagnosis of anaemia;
- Understand what each investigation specifically examines;
- Appreciate the significance of each test.
be representative of the patient’s true values. Paediatric samples are often obtained by prick ing the skin and squeezing out blood. Undue pressure may either artificially raise the haemoglobin (local haemocongestion) or lower it (tissue fluid). Adequate training in obtaining samples and ongoing practice under supervision are therefore imperative to ensure expertise in this area.

Storage

Everything that has been done to obtain an appropriate well-labelled sample is nullified if the sample is not stored and transported appropriately. If it cannot be refrigerated the sample should be kept in a cool, preferably dark, place and delivered to the laboratory as soon as possible.

Even in samples stored at room temperature (20°C) for 48 hours the numerical values are quite stable. The blood film, however, will be unreportable and other important values are affected. In practice, it should be possible for a sample to reach the laboratory within 24 hours.

The laboratory

A modern diagnostic laboratory is organised into a series of disciplines including clinical chemistry and haematology. Most tests are now automated. This saves on costs, but more importantly greatly improves the consistency of results. A typical full blood count report includes as many as 29 items of numerical data along with indications of the morphology of the blood cells. These computer-generated reports also frequently include flags indicating which results are outside set ranges.

By definition 95 per cent of all results from a healthy group of individuals will fall within the normal range. Therefore, 1 in 20 healthy individuals is expected to have a result outside the ‘normal’ range – such a result is not inherently indicative of disease.

An alternative set of values is the reference range – this is a population-specific normal range and would contain 95 per cent of the results for that population.

Other than the haemoglobin (Hb) values recommended by the World Health Organization to define anaemia, normal ranges are not cited. They are usually printed on laboratory reports and it is preferable, if there is any variation, to use the reference or normal ranges that are cited by your local laboratory.

The investigations

Haemoglobin

Haemoglobin (Hb) is an iron-containing pigment, which binds to and transports oxygen. Haemoglobin levels are determined by measuring the colour intensity of a blood sample in which the red cells have been lysed (broken open).

This is compared with an internal machine standard and the result is reported as either g/L or g/dl. The official SI unit for Hb is g/L but the g/dl notation is probably still more commonly used – values in g/dl are exactly 10 times smaller than g/L; an Hb of 150g/L is equal to 15g/dl.

Although other definitions are encountered, a reduced Hb level is the defining element of anaemia. The WHO defines anaemia as: ‘a haemoglobin (Hb) concentration in blood that is below the expected value, when age, gender, pregnancy and certain environmental factors, such as altitude, are taken into account’ (WHO, 2001).

The WHO recommends that normal ranges, (Table 1), should be used as the basis for investigation and treatment of individual patients. Reference ranges may be of value in determining the incidence and the impact of anaemia within a given population. However, they should not be used clinically.

The National Blood Service uses a simple screening test for anaemia, which relies on the specific gravity of a drop of blood placed in a copper sulphate solution.

If a potential donor fails this test a full blood count is done to confirm the result and provide further information. If necessary the donor’s GP will be contacted.

A low Hb value is pathognomonic of anaemia but offers no information regarding aetiology. Further investigations are then required in order to discover the cause of the anaemia and to determine its subsequent treatment.

## Table 1. The normal ranges stated by WHO

<table>
<thead>
<tr>
<th>Age/gender</th>
<th>Hb (g/dl)</th>
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<tbody>
<tr>
<td>Birth (full term)</td>
<td>13.5 to 18.5</td>
</tr>
<tr>
<td>Children: 2–6 months</td>
<td>9.5 to 13.5</td>
</tr>
<tr>
<td>Children: 6 months–6 years</td>
<td>11.0 to 14.0</td>
</tr>
<tr>
<td>Children: 6–12 years</td>
<td>11.5 to 15.5</td>
</tr>
<tr>
<td>Adult males</td>
<td>13.0 to 17.0</td>
</tr>
<tr>
<td>Adult females: non-pregnant</td>
<td>12.0 to 15.0</td>
</tr>
<tr>
<td>Adult females: pregnant</td>
<td></td>
</tr>
<tr>
<td>First trimester: 0–12 weeks</td>
<td>11.0 to 14.0</td>
</tr>
<tr>
<td>Second trimester: 13–28 weeks</td>
<td>10.5 to 14.0</td>
</tr>
<tr>
<td>Third trimester: 29 weeks to term</td>
<td>11.0 to 14.0</td>
</tr>
</tbody>
</table>

This article has been double-blind peer-reviewed.

For related articles on this subject and links to relevant websites see www.nursingtimes.net

### REFERENCES


Use the following points to write a reflection for your PREP portfolio:

- Record you place of work and why this article is relevant to the patients for whom you care;
- Write about what this article has taught you about investigations for anaemia;
- Explain how you will use this new knowledge with your patients;
- Describe how you will follow up your learning.

### Red cell count (RBC)

The red cell count (RBC) is an estimation of the number of red cells present in 1ml of whole blood. The exact method used to count the red cells varies between different designs of automated cell counter. The normal red cell count varies according to age and sex. If performed on an electronic counter this is a very accurate estimation. The principle source of error is the quality of the blood sample submitted.

### Mean cell volume (MCV)

Mean cell volume (MCV) is a measurement of the average size of the red cells. The normal range of the MCV is between 80 and 99fl.

The initial classification of anaemia is typically based in part on the MCV. A reduced MCV is termed microcytosis. An increased MCV is called macrocytosis. Where the MCV is within normal limits the anaemia is termed normocytic.

### Packed cell volume (PCV)

The packed cell volume (PCV) is often referred to as the haematocrit. It measures what percentage of a given volume of blood is made up of red blood cells. On automated counters the PCV is usually a calculated value derived from the RBC and the MCV. The PCV is affected by many conditions other than anaemia. Certainly the PCV alone can never be used to diagnose anaemia. The PCV may be reported as a percentage or as litres/litre.

### Red cell indices

The red cell indices are not directly measured properties of the red cell. They are values that are calculated from the results of the automated cell count. The most widely used are the mean cell haemoglobin (MCH) and the mean cell haemoglobin concentration (MCHC). The MCH is a calculated value based on the RBC and the Hb level. It is the amount of haemoglobin in an average red cell and is measured in picograms. The MCHC is again a calculated value based on the Hb and the PCV. It reflects the extent to which the red cell is ‘packed’ with haemoglobin – the units are g/dl.

### Reticulocyte count

Normal red cells undergo a maturation process within the bone marrow. During this process the cells shed their nuclei and all cytoplasmic structures concerned with protein synthesis (a mature RBC cannot synthesise proteins).

A small percentage of relatively immature RBCs are normally present in circulating blood. These have shed their nuclei but they still retain ribonucleic acid (RNA). They are known as reticulocytes.

### Category Common Causes

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### TABLE 2. MORPHOLOGICAL CLASSIFICATION

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and they offer a useful indirect index of marrow erythropoiesis (red cell formation). Reticulocytes may be visible in a routine blood film as large, slightly blue-staining, RBCs. To count reticulocytes requires a special stain – many modern haematology analysers can offer an automated reticulocyte count.

Manual counts (by microscopy) are usually reported as percentages (of all RBCs). A correction must be applied where anaemia is severe. Automated counts are typically reported as absolute numbers of reticulocytes per unit volume, reflecting the greater accuracy of such counts.

**Morphological classification of anaemia**

The most clinically useful initial classification of anaemia is based on the MCV and the MCH (Table 2).

**Test significance**

The defining feature of anaemia is a reduction in the circulating haemoglobin level. Anaemia can be found variously defined as:

- A reduced number of red cells;
- A reduced Hb level.

The correlation between clinical features and Hb levels depends to a large extent on whether the anaemia is acute or chronic in onset.

A woman whose Hb drops from 15g/dl to 12g/dl (both within normal limits) as a result of a brisk haemorrhage will show marked clinical features of anaemia. An older patient whose Hb has gradually declined to 4g/dl or 5g/dl as a result of nutritional deficiencies may show only mild clinical features. Haemodilution will eventually lead to a decrease in the RBC, Hb and associated parameters, as the body attempts to restore circulating volume and hence cardiac output.

Red cell values will not change in the short term. The RBC value is necessary for calculation of the PCV and red cell indices.

**Different types of anaemia**

Classification of anaemia as microcytic, normocytic or macrocytic is the first stage in the process of determining the type of anaemia and its cause.

**Microcytic anaemia**

The most common cause of microcytic anaemia is iron deficiency. In iron deficiency red cells are small but normal in shape. Sometimes elongated ‘pencil cells’ may be present.

When a premenopausal female presents with mild iron deficiency it is reasonable to assume this is due to menstrual blood loss and to institute a trial of iron therapy without further investigation. In older women and men iron deficiency due to dietary insufficiency is uncommon and a search should be instituted for occult sources of blood loss. Patients over 50 with iron-deficiency anaemia (IDA) are 31 times more likely to have a gastrointestinal malignancy (Ioannou et al, 2002). Microcytosis may be caused by haemoglobinopathy – an inherited abnormality of Hb production. This is rare in northern Europeans. The most common cause of the haemoglobinopathies are the thalassaemias and sickle cell anaemia.

Possibly the most useful test for iron deficiency is serum ferritin levels (Guyatt et al, 1992; Foret, 2002). However, there may be variations locally in the preferred testing algorithm.

**Normocytic anaemia**

Anaemia of chronic disease is the most common form of normocytic anaemia, which, in turn, is the most frequently encountered type of anaemia (Brill and Baumgardner, 2000). Anaemia of chronic disease may be microcytic – there are no pathognomonic features in the blood count or the blood film. It is a diagnosis of exclusion. Initially, nearly all anaemias are normocytic – only with time do they become microcytic or macrocytic. Normocytic anaemia is a result either of red cell destruction or loss (haemorrhage or haemolysis) or of deficient red cell production (aplasia, anaemia of chronic disease).

**Macrocytic anaemia**

Macrocytic anaemia may be caused by liver disease or by certain nutritional anaemias. The blood appearances can offer strong indications as to which is the more likely cause.

In liver disease the RBC is typically enlarged but normal in morphology. This is in marked contrast to megaloblastic anaemia due to folate or B12 deficiency. In this cells called oval macrocytes are typically present. These, together with distinctive abnormal neutrophils, are the hallmark of megaloblastic anaemia.

Confirming that anaemia is megaloblastic and identifying the underlying cause requires specific investigations. (Oh and Brown, 2003).

**Conclusion**

Nurses in both the primary care setting and the ward setting need an effective knowledge of the relevant laboratory values and investigations of anaemia. Samples must be taken correctly to ensure accuracy and nurses need to have some understanding of the significance of the test results in the classification and correct management of this common condition.