East African Regional External Quality Assessment Scheme (EA-REQAS)
Learning Sheet Number Two
HAEMOGLOBIN ESTIMATION

INTRODUCTION

Haemoglobin is the red pigment inside red blood cells that carries oxygen from the lungs to the body tissues. Normal haemoglobin values are defined as those found in healthy populations. Abnormal haemoglobin levels include those that are too high (polycythaemia) or too low (anaemia).

Polycythaemia is uncommon and may be due to a response to decreased oxygen levels in blood, such as patients with chronic lung disease, congenital heart disease or heavy smokers, or in people living at high altitude (0.5 – 1.5 g/dl increase in haemoglobin for every 5000 feet above sea level). Polycythaemia may also occur in malignancies of the kidney, liver or bone marrow.

Anaemia is much more common and results from one or more of the following processes:
- Defective red cell production due to:
    - Insufficient essential nutrients in the diet or increased utilisation, such as in pregnancy, lactation or rapid growth of premature infants
    - Acute infections, such as malaria; and chronic infections such as tuberculosis, visceral leishmaniasis
    - Chronic conditions, such as chronic renal failure
    - Bone marrow disorders such as aplastic anaemia, leukaemia. Aplastic anaemia may result from use of certain drugs, such as chloramphenicol
- Increased red cell destruction (haemolysis):
    - Parasitic diseases, such as malaria
    - Genetic conditions such as sickle-cell disease, glucose 6-phosphate dehyrogenase (G6PD) deficiency
    - Immune conditions, such as autoimmune haemolytic anaemia
- Chronic blood loss, such as from intestinal worm infestation, for example hookworm, from heavy menstrual flow or bleeding peptic ulcer

More than one cause of anaemia may be present at the same time.

Normal reference ranges for haemoglobin are:

Population	Hb g/dl
Men (adults)	13 – 18
Women (adults)	12 – 16
Women (pregnant)	11 – 14
Children (6 – 14 years)	12 – 14
Children (6 months – 6 years)	11 – 13
Neonates (up to 28 days)	12 – 16
Infants (full term at birth)	15 – 25

Haemoglobin estimation is performed to:
1. Confirm the presence of anaemia or polycythaemia
2. Monitor and follow up patients on treatment for anaemia or polycythaemia
3. Screen for anaemia in high risk groups, such as pregnant and lactating women, premature infants and children at the time of weaning
4. Assess blood donors prior to donation, or as part of routine medical examinations

SYMPTOMS AND SIGNS OF ANAEMIA AND POLYCYTHAEMIA

Symptoms of anaemia include shortness of breath during exercise, palpitations, dizziness, weakness and tiredness. Signs of anaemia include pallor of mucous membranes, nail beds, palms and soles, a rapid pulse and increased respiratory rate. Severe anaemia, especially if rapidly developing, may lead to heart failure.

In adults, signs of cardiac failure are: crackles in the lung bases, peripheral oedema, enlarged liver, raised jugular venous pressure

In infants, signs of cardiac failure are: grunting, intercostal or subcostal retractions, nasal flaring, increased heart and respiratory rate, enlarged liver, oedema

Symptoms and signs of anaemia may not correlate well with the haemoglobin level because the body has many ways of compensating for slowly developing anaemia. Children and infants are remarkably tolerant of anaemia.

Polycythaemia may cause headaches, dizziness, fatigue and drowsiness.

In addition to the signs and symptoms of anaemia and polycythaemia, clinicians should look for symptoms and signs of any underlying causes.

HAEMOGLOBIN ESTIMATION IN THE LABORATORY

Blood samples

Use capillary blood or venous blood anticoagulated with EDTA. Blood anticoagulated with EDTA can be used for up to 1 week if stored in the refrigerator at $4 - 8^{o}C$. Do not use sodium citrate solution as anticoagulant as it dilutes the blood.

Methods of haemoglobin estimation

There are three general methods for measuring haemoglobin concentration in blood:
- Visual comparison of colour
- Colorimetric (manual) methods
- Electronic blood cell analysers

Visual methods include:
- Tallqvist or WHO Colour Scale (filter paper)
- Sahli method (acid haematin)
- Lovibond comparator (oxyhaemoglobin)
- Grey wedge (Spencer method or BMS photometer)

In the visual methods, the observer matches the colour of the sample against a series of standards, either directly or after adding a chemical or lysing agent to the blood. These methods are subjective, that is, the results depend on the opinion of the observer. Therefore these methods are not recommended.

Colorimetric (manual) methods using include:

- Haemiglobincyanide (formerly cyanmethaemoglobin) method
- Oxyhaemoglobin method
- Alkaline haematin method

In addition, manual colorimetric methods include the Hemocue® that is supplied with disposable cuvettes containing a lysing agent; and the Mission® haemoglobinometer that is supplied with reagent nitrocellulose strips.

The alkaline haematin method performs in a similar way to the haemiglobincyanide method but uses no toxic reagents. In addition primary stable standards for alkaline haematin are available.

Electronic blood cell analysers use a photometric method and perform haemoglobin estimation automatically.

Comparison of methods of haemoglobin estimation[1,2]

	Visual methods	Colorimetric (manual)	Electronic blood cell analyser
Accuracy	Not accurate (± 2g/dl)	Accurate (± 0.5g/dl)	Accurate (± 0.5g/dl)
Precision	Not precise	Precise	Precise
Test time	Varying (3-20 minutes)	Varying (2-15 minutes)	Quickest (2-5minutes)
Source of power	No power required	Electricity, battery	Electricity
Equipment	Inexpensive	Moderately costly (<500 USD)	Expensive (>10,000 USD)
Reagents per test	Relatively cheap	More expensive (US$ 0.2 – 0.4)	More expensive (US$ >1)
Anaemia screening	Appropriate	Appropriate	Appropriate
Anaemia diagnosis	Not appropriate	Appropriate	Appropriate
Follow up treatment	Not useful	Appropriate	Appropriate
Standards for quality control	None	Not available for oxyhaemoglobin	Standards available

Procedural notes

- When using a comparator method, always read the results in a good light
- Never use expired reagents
- Always follow the manufacturer's instructions carefully for reagent preparation and storage, and for the test procedure
- When using a manual colorimeter, ensure the correct filter is in place for haemoglobin estimation

SOURCES OF ERRORS IN HAEMOGLOBIN ESTIMATION

1.   Sampling problems:
   - Failure to wipe off excess blood from the outside of the pipette or pipette tips
   - Failure to measure the blood accurately, such as use of chipped pipettes, pipettes coated with plasma proteins, or re-using pipette tips
   - Inadequate rinsing of the pipette
   - Improperly mixed samples

2.   Clerical errors:
   - Mislabelling, interchanging results

3.   Faulty reagents:
   - Improperly prepared, poorly storage, expired reagents
   - Use of reagents intended for other analysers

4.   Faulty or malfunctioning equipment:
   - Wrongly calibrated pipettes
   - Uncalibrated colorimeter

QUALITY CONTROL OF HAEMOGLOBIN ESTIMATION

Always use commercially prepared control samples when operating manual colorimeters and autoanalysers. Control samples should be run in duplicate, on a daily basis. If commercial control samples are not available, a blood sample of known concentration can be used for up to 1 week if kept refrigerated; however use of a blood sample will not detect drift and therefore a sample that has been measured accurately on another instrument should be obtained approximately every 2 -3 months.

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