Anemia and hemoglobin testing

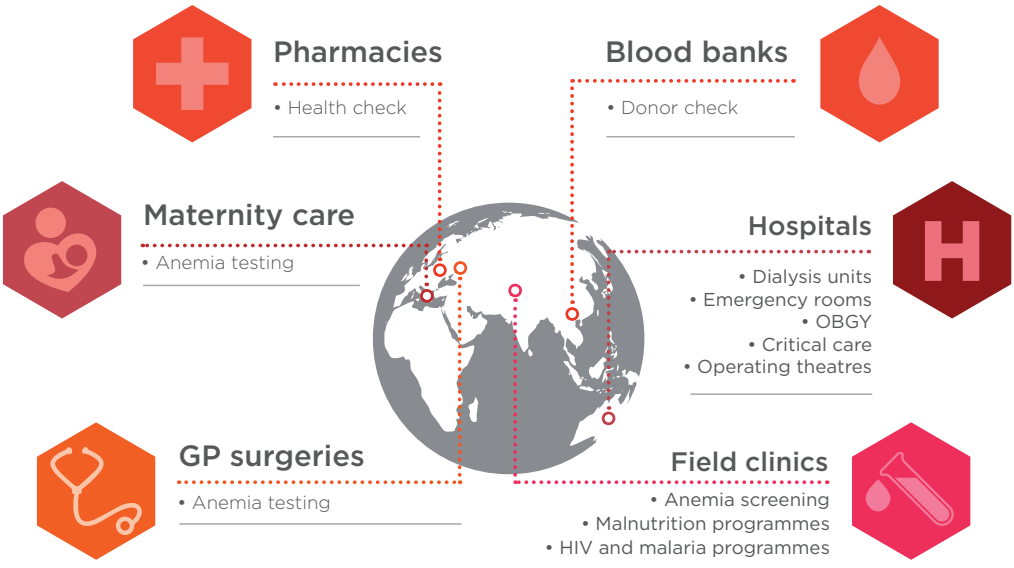
EKF | Hematology

Our mission

EKF Hematology aims to make blood donation and anemia screening easier, more affordable and more accessible than ever before.

Our diagnostic tests deliver fast and reliable results for hemoglobin and hematocrit that provide both practitioner and patient with the information they need to make clinical or lifestyle decisions in seconds.

Contents



1 What is anemia?

Anemia is a condition in which the number of healthy red blood cells or the availability of hemoglobin falls below the body's physiologic needs.

Hemoglobin (Hb) is a protein based component and is the main part of red blood cells. It binds oxygen to transport around the body and ensures sufficient supply to tissues and organs. Hemoglobin also helps in the transportation of carbon dioxide and hydrogen ions back to the lungs.

The individual physiological need depends on several factors like age, gender, altitude, smoking behavior and different stages of pregnancy. The volume percentage of red blood cells in a blood sample is called hematocrit (Hct), also known as packed cell volume (PCV).

Hemoglobin or hematocrit tests are the main blood tests used to diagnose anemia. Anemia can be caused by poor nutrition or various diseases.

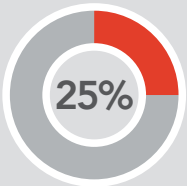
Table 1.0: Hemoglobin (Hb) level used to define anemia

Age or gender group	Hb level (g/dL)	Hb level (g/L)
Children below 5 years of age	≤ 11.0	≤ 110
Children 5 - 11 years of age	≤ 11.5	≤ 115
Children 12 - 14 years of age	≤ 12.0	≤ 120
Non-pregnant women (15 years of age and above)	≤ 12.0	≤ 120
Pregnant women	≤ 11.0	≤ 110
Men (15 years of age and above)	≤ 13.0	≤ 130

Adapted from[1)]

Did you know?

Anemia is the most common disorder of the blood, affecting about 25% of the global population



2 Causes of anemia

There are many different types of anemia that can be divided into three groups:

- Anemia caused by blood loss
- Anemia caused by decreased or faulty red blood cell production
- Anemia caused by destruction of red blood cells

The most common cause of anemia is iron deficiency. The hemoglobin protein is formed from four subunits, each with a cofactor called a heme group that has an iron molecule at its center. Iron is the main component that actually binds to oxygen, so each hemoglobin molecule is able to carry four molecules of O_2.

Most of the body's iron (ca. 70%) is found in hemoglobin and in the myoglobin of muscle cells, whereas about 25% is stored as ferritin. The average adult male has about 1,000 mg of stored iron, enough for about three years. Women on average have about 300 mg, enough for about six months.

When iron intake is chronically low, stores can become depleted and hemoglobin levels decrease.

Other nutritional deficiencies causing or contributing to anemia are insufficient supply of folate (folic acid), vitamin B12, vitamin A, riboflavin and copper.

A number of diseases such as acute and chronic inflammation, cancer, malaria, parasitic infection, HIV and genetic defects that result in abnormal structures of hemoglobin, like in sickle-cell disease or shortened life-time of red blood cells, like in Thalassemia, can be the underlying cause of anemia.

Did you know?

Iron deficiency is the most common and widespread nutritional disorder in the world and the only nutrient deficiency with significant prevalence also in industralized countries.

3 Symptoms and health consequences

The main symptoms of anemia are:

- Fatigue (the most common symptom)
- Shortness of breath
- Dizziness and headaches
- Cold hands and feet
- Pale skin and chest pain

In mild to moderate anemia these clinical symptoms may be less visible. The clinical effects of anemia also depend on its duration and severity. In acute anemia the body does not have enough time to make the necessary physiologic adjustments, and the symptoms are more likely to be pronounced and dramatic.

In contrast, when anemia develops gradually, the body is able to adjust by increasing cardiac output, shunting of blood to vital organs, enhancing oxygen release to the tissues and increasing erythropoietin (EPO) to stimulate red blood cell production.

Anemia reduces an individuals' wellbeing, physical productivity and work performance. It has a negative impact on development and the learning of children. Maternal anemia is associated with mortality and morbidity in the mother and baby, including the risk of miscarriages, stillbirths, prematurity and low birth weight.

Did you know?

Timely treatment can restore personal health and raise national productivity levels by as much as 20% in developing countries [2)]

4 Patients at risk

Women in the childbearing years are particularly susceptible to iron-deficiency anemia because of the blood loss from menstruation and the increased blood supply demands during pregnancy.

The maternal blood volume needs to increase by 40-50% during **pregnancy** to supply the fetus. The increased need of iron and folic acid can often not be covered by regular nutrition.

Hemoglobin concentrations even in healthy, iron-sufficient women decline during the first trimester in pregnancy reaching their lowest point in the second trimester, diminishing by ca. 0.5 g/dL (5 g/L). Thereafter, hemoglobin levels begin to rise again in the third trimester.

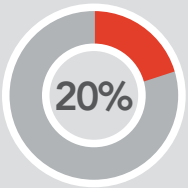
In developing countries the coincidence of malnutrition, infections and early as well as frequent pregnancies, increases the rate of anemia up to 75% in women.

Anemia can also be caused by Postpartum hemorrhage (PPH) - an estimated vaginal blood loss of greater than 500 mL within 24 hours after vaginal delivery or greater than 1,000 mL after cesarean delivery. Maintaining adequate hemoglobin and hematocrit levels by taking vitamin and iron supplements in the antepartum period is important for women with known risk of developing PPH. Monitoring hemoglobin and hematocrit closely is also essential in the course of PPH including the assessment of transfusion needs.

Globally, the highest prevalence of anemia is found in **preschool-age children** (47%). Children have increasing hemoglobin needs to manage their growth. They may suffer from inherited hemoglobin malformations (e. g. Sickle cell, Thalassemia), parasitic infections or poor nutrition. The highest proportion of preschool-age children affected by anemia live in Africa (67.6%), while the greatest number affected are in South-East Asia (115.3 million).[3)]

Did you know?

Anemia contributes to 20% of all maternal deaths.[2)]



In **adolescent girls** anemia can be caused by heavy and prolonged menstrual bleeding. Several treatment options, including iron supplementation, are available to manage this condition and improve the adolescent's quality of life.

In **toddlers** iron deficiency anemia can be a result of high milk consumption. Milk provides calcium and vitamin D but is low in iron and it inhibits the absorption of iron in the body. From the age of 6 months, all infants and toddlers should receive iron-rich (complementary) foods, including meat products and/or iron-fortified foods. Unmodified cow's milk should not be fed as the main milk drink to infants before the age of 12 months and intake should be limited to <500 mL/day in toddlers.[14)]

Patients with blood loss from surgery or an injury need a close surveillance of their hemoglobin levels in the course of their treatment to avoid an anemic situation.

Other risk factors for anemia are long-term or serious illnesses such as kidney disease, cancer, diabetes, rheumatoid arthritis, HIV/AIDS, inflammatory bowel disease (including Crohn's disease), liver disease, heart failure and thyroid disease as well as long-term infections or a family history of inherited anemia.[13)] Gastrointestinal (GI) bleeding is a common clinical problem and can vary from a massive life threatening hemorrhage to a slow, often unnoticed, chronic blood loss. This can result in iron deficiency anemia. Typical causes of GI bleeding are ulceration, esophageal varices or varicose veins, a hiatal hernia, a colon polyp or colorectal cancer, tumors and inflammation.

Anti-inflammatory medications (in particular aspirin or other arthritis drugs) and thinning of the blood from certain medications like Warfarin can aggravate upper GI bleeding.

5 Blood donation

Donating blood is a life-saving aid for many patients, not only in emergency situations but also for those depending on long-term treatments.

Recent improvements in the treatment of cancer patients and the progresses in "minimally invasive" surgery decreased the demand for blood as did the adjustment of transfusion thresholds based on latest medical findings.

Additionally, patients undergoing scheduled surgery are treated for anemia in the weeks before to minimize the need for transfusions. This requires close monitoring of hemoglobin levels in the pre-surgery period.

Globally, there is still a strong discrepancy between the coverage of blood transfusion needs in high, mid and low income countries. About 108 million blood donations are collected worldwide.

Today, more than half of these are collected in high income countries, representing only 18% of the world's population. In low income countries the donation rate is only about 10% of what is commonly found in high income countries.

Encouragingly, a noticeable increase of voluntary unpaid blood donations in South East Asia (78%) and Africa (51%) was seen between 2004 and 2012. The maximum increase in absolute numbers was reported in the Western Pacific Region.[4)]

The limited life-time of blood products (up to 42 days for red cells and around five days for platelets) means that hospitals must plan their stock carefully in order not to run out of any category. Regular registered donors help to ensure the availability according to the hospitals requirements. But during vacation periods or in the case of unexpected events blood banks may still need to issue emergency appeals to their donors.

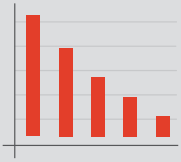
Pre-donation hemoglobin testing is an integral part of donor assessment in many countries. In healthy donors the amount of stored iron is sufficient to build up enough hemoglobin and consequently to replace the red blood cells withdrawn during blood donation. But if hemoglobin is already low before the donation there is a risk of inducing iron deficiency anemia.

Most national guidelines state that donors with hemoglobin levels below 13.5 g/dL for men and 12.5 g/dL for women should not donate blood. Some countries such as the US for example are using 12.5 g/dL for both men and women, or a hematocrit of 38% as a lower limit.

The main reason for donor deferral is low hemoglobin (Hb) with a varying deferral rate depending on the setting or region. The vast majority of deferrals occurs in women.

Did you know?

Highly accurate, quantitative hemoglobin measurement can lead to a significant reduction in donor deferral rate and ensures donor safety at the same time.[5)]



6 Treatment of anemia

Following the initial diagnosis the causes of anemia must be identified in the individual patient to enable a successful treatment of the disease. Considering the often multifactorial nature of anemia this may require an integrated approach.

In the case of iron deficiency anemia (IDA), an increase of iron intake as well as enhancement of iron absorption will be aimed for. Depending on the degree of anemia and the living conditions of the patient this may include:

Dietary diversification including iron-rich foods

There are two types of iron: heme iron from animal sources and non-heme iron from plant sources. While both are needed for a healthy diet, the absorption of heme iron is much greater than from non-heme iron which must be converted before it can be absorbed.

Examples of iron-rich food are:

- Red meat
- Fish and sea food
- Nuts (including peanut butter) and seeds
- Pulses and beans, especially chickpeas, soybeans and lentils
- Brown rice
- Whole-meal or brown bread
- Leafy green vegetables such as broccoli and spinach
- Dried fruit, especially dried apricots, raisins and prunes
- Tofu
- Dark chocolate

Other food components inhibit the uptake of iron and should be avoided during meals:

- Consumption of high levels of calcium (>40 mg) like milk
- Tea, coffee, peppermint and chamomile

Food fortification

Fortification of cereals, wheat and maize flours containing iron, folic acid and other micronutrients is advised in settings with a high occurrence of IDA where these foods are major staples.

Iron supplementation

In persistent IDA, in pregnant and lactating women and in poor settings, iron supplementation with tablets or formulas may be the only way of managing anemia successfully.

Latest findings indicate that besides its positive effect of enhancing hemoglobin production, there is a potential side effect of high iron levels on the immune system including promotion of microbial growth.[6)7)] This may especially affect patients with existing immune deficits or underlying infections.

It seems reasonable that iron supplementation should be monitored closely for its need, dose effectiveness and side effects in these patient groups.

Prevention and control of other nutritional deficiencies, such as vitamin B12, folate and vitamin A should accompany the treatment of IDA.

An essential part of anemia treatment and prevention is education. IDA is in many instances linked to poverty and low social status. National and NGO supported anemia screening programs combine treatment, prevention and education.

Did you know?

Vitamin C enhances the uptake of iron from food components. Combining iron and vitamin C rich food or a glass of fruit juice with your meal helps your body use the iron from these foods.

7 Methods of testing Hb/Hct

Hemoglobin and hematocrit can be measured by a variety of methodologies. Point-of-care (POC) testing in hematology has continued to grow in popularity; the uptake throughout the world and rapid improvements in technology have led to the development of several devices.

The measurement of hemoglobin is the most used parameter in POC hematology.[8)]

Cyanmethemoglobin (HiCN) – the reference method

The cyanmethemoglobin method works on the principle of conversion of hemoglobin to cyanmethemoglobin by the addition of potassium cyanide and ferricyanide whose absorbance is measured at 540 nm in a photometer against a standard solution.[9)]

Based on the first initiatives to standardize this method by Drabkin and Austin the "International Committee for Standardization in Haematology" (ICSH) was founded in 1964. ICSH was given the task to develop recommendations for the measurement of hemoglobin which were then published in 1967. The CLSI (at that time NCCLS) converted these recommendations into a formal standard named NCCLS H15-A3. Both NCCLS and ICSH use the same method.

Today the HiCN method is still in routine use in laboratories in rural countries but being time consuming and dependent on cyanide (toxic) agents it is predominantely used as a reference method for the calibration of modern POC hemoglobin devices and lab analyzers.

Standardized, stable reference material is used for the calibration and ensures traceability of POC results back to the international reference method.

Vanzetti´s azide methemoglobin method

The first generation of portable POC hemoglobin devices with single use, dry reagent cuvettes operates based on a modification of Vanzetti´s azide methemoglobin method (HemoCue® 201, EKF Hemo Control).

The blood is drawn into the cuvette by capillary action and the walls of the red blood are destroyed (hemolysed) by the reagent. The free hemoglobin is oxidized to methemoglobin which is then converted into azide methemoglobin, a stable colored complex. This complex is then measured photometrically at 570 nm and at 880 nm for compensation of turbidity. A measurement takes between 15 – 60 seconds, depending on the hemoglobin concentration.

All hemoglobin variants are converted (except SulfHb) and stable results are obtained during the ten minutes after filling the cuvette, shown in repetitive measurements.

A high sensitivity and specificity has been described for this method [9] and today it is the most common and established POC method for measuring hemoglobin in clinical as well as in blood collection settings.

Reagent-less methods

A constant draw-back of the azide methemoglobin method is the susceptibility of the reagent to humidity, especially in challenging climate conditions. Cuvettes need to be stored in a carefully closed canister with desiccant and removed directly before usage. The shelf-life after opening the canister is limited to three months.

POC devices using reagent-less cuvettes were developed to overcome these limitations. The first device using reagent-less cuvettes was the HemoCue® 301, launched in 2006. It measures the absorbance of whole blood photometrically at a wavelength of 506 nm, and at 880 nm for turbidity compensation. At 506 nm the two main hemoglobin derivatives, oxy-hemoglobin (HbO_2) and deoxy-hemoglobin (Hb) have their isosbestic point, a wavelength in which the absorbance of two or more species are the same.

7 Methods of testing Hb/Hct

DiaSpect, now an EKF Diagnostics company, developed a different technology: a white LED is flashed briefly and the light beam passes through the sample to a specially designed optical sensor element. The sensor detects the absorbance of the blood sample at a broad wavelength range to get an overall picture of the absorbance spectrum. A patented light trap prevents scattered light from arriving at the sensor. This is essential in order to obtain accurate results when measuring non-hemolyzed blood.

Additional advantages besides the use of reagent-less cuvettes with long shelf-lives (up to 2.5 years) are ease of use, a rapid measurement time of just about one second and a long standing time of the in-built rechargeable battery of 40 days in use.

Non-invasive methods

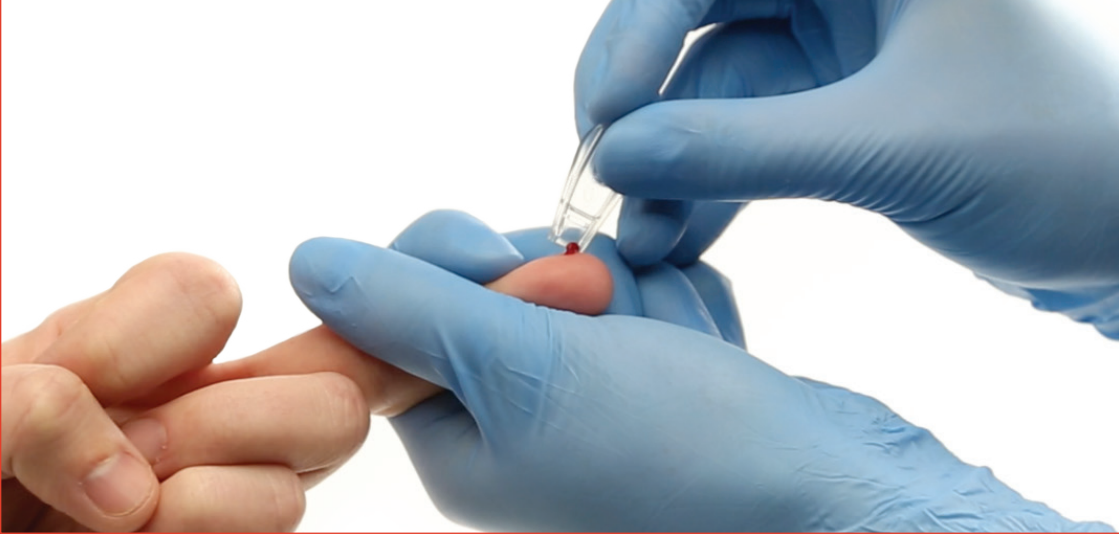
Recently, non-invasive methods have become commercially available using near-infrared spectroscopy to identify the spectral pattern of Hb in an underlying blood vessel and derive a measurement of Hb concentration. Other non-invasive devices use white light and capture the reflected transmission data in order to measure Hb levels in tissue capillaries or multiple wavelength light absorption to calculate the Hb concentration. A finger-clip or ring is used to apply the sensor.

Given the advantage of obsolescence of finger-sticks and the option for more frequent measurements in clinical settings, the literature remains unclear about the precision and accuracy of the current non-invasive hemoglobin monitors. In practical use, patient movement, nail polish, skin color or ambient light have been shown to influence the measurement.[10)]

Other methods of Hb / Hct testing - Visual methods:

Sahli´s method

Sahli's manual hemoglobinometer contains a comparator, hemoglobin tube, hemoglobin pipette and stirrer. Hemoglobin is converted to acid hematin by the action of HCL. The acid hematin solution is further diluted until its color matches exactly with that of the



permanent standard of the comparator block. The hemoglobin concentration is read directly from the calibration tube. In developing countries like India, Sahli's method, invented in 1902, is still the most common method used for hemoglobin estimation. The method is simple and cheap but rather inaccurate. The color developed is unstable and must be read after 10 minutes standing. There is inter-observer variability and the use of manual pipetting makes it prone to errors and there is no international standard.[9)]

WHO color scale (HbCS)

The HbCS relies on comparing the color of a drop of blood absorbed on a test strip of special chromatography paper with standard colors on a laminated card displayed in increments of 2g/dL (20 g/L).

The cost per test is very low and apart from materials for taking the blood sample no technical equipment is needed. However, the visual comparison is susceptible to inter-observer variability and a low sensitivity has been found in some studies.[9)]

Copper sulphate (CuSO4)

The copper sulphate method is mostly used to ensure a certain Hb level for blood donation. It is based on the hemoglobin dependent gravity of blood. A blood droplet is allowed to fall into copper sulphate solution of a specific gravity, equivalent to that of blood with the cut-off hemoglobin level, e.g. 12.5 g/dL (125 g/L). If the drop of blood sinks to the bottom in an acceptable amount of time, the donor qualifies. If the drop of blood floats or takes too long to sink, the donor is deferred.

The CuSO4 method is hampered by a lack of quality control, problems with disposal of the biohazardous solutions and erroneous results in individuals with a very high serum protein concentration. Furthermore, there are concerns that the CuSO4 method might give falsely high deferral (i.e. 'false-fail') rates for donors, particularly in women.[8)] It is a common practice in many settings to re-test donors who failed in the CuSO4 test with a quantitative measurement.

7 Methods of testing Hb/Hct

Quantitative methods:

Hematology analyzer/CBC

Automated hematology analyzers can provide a high precision, enable high sample through-puts and analyze a number of different types of red and white blood cells (3-part, 5-part or 7-part differential), blood platelets, hemoglobin and hematocrit levels from the same blood sample.

But the investment costs for the analyzer are high and it may not be suitable outside a laboratory environment. Skilled laboratory personal, regular maintenance and stable climate conditions are needed to operate hematology analyzers. In most instances, the sample needs to be sent to the laboratory causing longer turn-around times for the results.

Blood gas analyzer (BGA)

Blood gas analyzers are used to measure combinations of pH, blood gas (i.e. pCO_2 and pO_2), electrolytes and metabolites parameters from whole blood samples, mainly from arterial blood.

They are commonly used in critical care units, operating theatres, delivery wards and emergency rooms. Technical improvements like ready-to-use sensor cassettes and solution packs have made the usage of BGA much more convenient but maintenance is still required. Recently, hand-held devices with single use cartridges have become available but the cost per test is high compared to a POC hemoglobin meter – if Hb is the primary interest. Besides, some cartridges require cool storage and pre-warming before the test can be done.

Microhematocrit centrifuge

Microhematocrit centrifuges are used to determine the blood's hematocrit—the ratio of red cell volume to whole blood volume, expressed as a decimal, a fraction, or a percentage. Diagnosis of anemia or determination of donor eligibility based on hematocrit is a common practice in many countries. A capillary of blood is spun and the separated red blood cell and plasma segments are measured. In some applications the plasma from the capillary is used for further analysis, e .g. for proteins prior to plasma donation.

A conversion factor of x 3 can be used to calculate the approximate hematocrit level from a hemoglobin measurement and vice versa.

8 Factors influencing the measurement

Variability in reported hemoglobin values can be caused by a number of physiological factors and sampling mistakes. It is of great importance to establish a standardized procedure when measuring hemoglobin.

Physiological factors

Gender	For a given finger stick result, the expected venous Hb value is 0.5 to 0.8 g/dL lower for women compared to men. [11]
Source of sample	Capillary blood has higher Hb than venous blood, especially in women and in men with severe iron depletion (median +0.67 g/dL or +6.7 g/L for iron-depleted women to -0.1 g/dL or -1 g/L for iron-repleted men). [11] Venous blood has a slightly higher Hb than arterial blood.
Sampling site	Ear stick sampling has been used in the past but has been shown to produce values that are higher than venous and finger stick values. Finger stick sampling has been shown to more closely approximate venous Hb values.[12]
Tourniquet use	Tourniquet use longer than 30 seconds increases venous hemoglobin value. [11]
Body position	Hb is higher in blood samples from standing subjects than in samples from sitting or supine subjects. For example an increase of up to 9% after 15 minutes of standing or a decrease of 2.4-2.7 % when moving from a standing to a seated position can be seen in venous hemoglobin values. [11]
Diurnal variation	Hb tends to be higher in the morning and decreases throughout the day. [11]
Dehydration	Loss of plasma volume for example, due to transpiration or insufficient fluid uptake causes increased hematocrit and hemoglobin values.
Altitude	The normal hemoglobin concentration increases at high altitudes (>1,500 m) to compensate for the lower concentration of oxygen in the air. This effect becomes more pronounced with increasing altitudes and should be corrected for when interpreting hemoglobin results .[1]

When carrying out comparative Hb testing for studies or evaluations, the samples should be taken under identical conditions.

8 Factors influencing the measurement

Capillary sampling technique

Both accuracy and reliability of hemoglobin measurements can be affected by pre-analytical errors. Following a standardized procedure, as well as operator training and practice, is essential to obtain correct results especially from capillary sampling (finger sticks).

Potential sources of error are:

Choice of lancet	The lancet must make a sufficiently deep puncture to ensure an adequate flow of blood (1.85 to 2.25mm is recommended, depending on the thickness of the skin).
Selection of puncture site	The middle or ring finger should be used, ideally of the non-dominant hand, as they are generally less calloused and less sensitive to pain compared to the index finger or thumb. The thumb should also be avoided due to its pulse (arterial presence). In the fifth finger the distance between the skin surface and the bone is too small. The puncture should be made slightly off center from the central, fleshy portion of the fingertip – near the side, but not on the very side of the finger. The hand must be warm and relaxed. The patient must not wear a ring on the finger as this may obstruct the blood circulation.
Cleaning & disinfection	After cleaning and disinfection, the puncture site must be dried completely. Remnants of alcohol solution will dilute the blood and cause false low readings.
Puncture	The finger should be supported by the operator´s hand. It can be massaged gently before and after the puncture to stimulate blood circulation. Maintaining a light pressure in the moment of the puncture helps to achieve a good penetration.
Capillary flow	The first 2-3 drops of blood should be wiped away using a clean gauze pad. Thinning and clotting of the blood must be avoided as it causes incorrect results. A good capillary flow is normally found within 30-45 seconds after the puncture. The 3rd or 4th drop of blood should be used to fill the cuvette for the hemoglobin measurement. The drop must be big enough to fill the cuvette completely. Incomplete filling or air bubbles cause false results. The finger must not be squeezed hard or "milked" to increase the size of the drop as this will dilute the sample with interstitial fluid.

9 References and units

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Unit conversions for Hb and approximate Hct values			
g/dL	g/L	mmol/L	~ %Hct
2.0	20	1.2	6
4.0	40	2.5	12
6.0	60	3.7	18
7.0	70	4.3	21
8.0	80	5.0	24
9.0	90	5.6	26
10.0	100	6.2	29
11.0	110	6.8	32
11.5	115	7.1	34
12.0	120	7.4	35
12.5	125	7.8	37
13.0	130	8.1	38
14.0	140	8.7	41
15.0	150	9.3	44
16.0	160	9.9	47
17.0	170	10.6	50
18.0	180	11.2	53
20.0	200	12.4	59
22.0	220	13.7	65
24.0	240	14.9	71

EKF Diagnostics Holdings plc
Avon House, 19 Stanwell Road, Penarth, Cardiff CF64 2EZ

Hematology

Telephone. +44 (0)29 20 710570
Email. info@ekfdiagnostics.com
Web. www.ekfdiagnostics.com

Rev EN EU 0.0-11.15