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Clinical Hematology

- **Introduction**
- **Physical features**
- **composition**
- **Hematological tests**

-collection blood
-routine hematological tests in hospitals

- **Electronic hematology analyzers**
- **Commonly used anticoagulants**

Introduction
Hematopoiesis – the generation of blood cells that proceeds mainly in the bone marrow - is a well-controlled process constantly occurring throughout the live of the mammalian organism. Generally, blood cells are relatively short-lived cells with a life span ranging from few hours to several weeks causing the need for a sustained replenishment of functional erythroid, lymphoid and myeloid cells.

Hematology (Greek Haema = Blood; logy = Study of). Hematology is the branch of medical science that deals with the study of blood.

Physical features. The blood is denser and more viscous than water, slightly alkaline, sticky to touch, and salty in taste. It clots on standing, leaving behind serum. The normal total circulating blood volume amounts to 8% of the body weight, i.e. 5–6 liters in an average adult male weighing 70kg, and 4–5 liters in a female. The interplay of various hormones that control salt and water excretion in the urine keep the blood volume remarkably constant.

Composition. Blood consists of 55% of watery liquid plasma that contains various proteins and other solutes dissolved in it. The rest 45% is the formed elements— mainly the red blood cells (RBCs) but also white blood cells (WBCs), and platelets (cell fragments). The RBCs are the most numerous (4.5–5.5 million/mm3) and are medium sized (7–8 µm). Next in number are platelets (2.5–4.5 lacs/mm3) and are the smallest (2–4 µm) in size. The WBCs number 4000–11000/mm3 and vary in size from

8 to 20 μm. The percentage of whole blood that is red cells is called ***hematocrit***, its value being 45.

Hematological tests. The experiments described in this section are carried out as routine hematological tests in hospitals and clinical laboratories for aiding in the diagnosis and prognosis of disease. Some tests (e.g. hemoglobin, cell counts, etc.) are simple enough, while others require some degree of practice and understanding.

collection of blood samples

1. Asepsis

The term asepsis refers to the condition of being free from septic or infectious material—bacteria, viruses, etc. In order to achieve asepsis, the following aspects need to be kept in mind:

A. Sterilization of equipment

All the instruments to be used for collecting blood—syringes, needles, lancets, and cotton and gauze swabs—should preferably be sterilized in an autoclave.

B. Cleaning/sterilizing of skin

At least 2–3 sterile cotton/gauze swabs soaked in 70% alcohol, methylated spirit, or ether should be used to clean and scrub the area. Cotton swabs are likely to leave fibers sticking to the skin and provide an undesirable contact, or they may appear as artifacts in a blood film.

C. Prevention of contamination.

Any material used for skin puncture, or the operator's hands may cause contamination. Therefore, once the site has been cleaned and dried, it should not be touched again. Care must be taken to prevent contamination until the puncture wound has effectively closed/healed.

2. The blood sample

The term "blood sample" refers to the small amount of blood—a few drops or a few milliliters—obtained from a person for the purpose of testing or investigations. These tests are carried out for aiding in diagnosis and/or prognosis of the disease or disorder.

A. Sources and amount of blood

i. Capillary blood. The skin and other tissues are richly supplied with capillaries, so when a drop or a few drops of blood are required, as for estimation of Hb, cell counts, BT and CT, blood films, micro chemical tests, etc, blood from a skin puncture (skin-prick) with a lancet or needle is adequate.

ii. Venous blood. When larger amounts (say, a few ml that cannot be obtained from a skin puncture) are needed as for complete hematological and biochemical investigations, venous blood is obtained with a syringe and needle by puncturing a superficial vein. In infants, venous blood may have to be taken from the femoral vein, or the frontal venous sinus.

iii. Arterial blood. When arterial blood is needed for special tests such as blood pH, gas levels, etc, an artery such as radial or femoral is punctured with a syringe and needle. This, however, is not a routine procedure.

iv. Cardiac catheterization. Blood from a heart chamber, taken through a cardiac catheter, may be required for special tests.

B. Containers for blood samples

A container is a receptacle into which blood is transferred from the syringe before sending it to the laboratory. Clean and dry 10 ml glass test tubes, collection bottles such as clean and dry 10 ml discarded medicine vials, glass bulbs, etc are the usual ones in use.

A container may or may not contain an anticoagulant depending on whether a sample of blood/plasma, or serum is required.

For a sample of whole blood or plasma. The blood is transferred to a container containing a suitable anticoagulant. This is to prevent clotting of blood.

For a sample of serum. No anticoagulant is used. The blood is allowed to clot in the container and serum is collected as described later.

3. Commonly used anticoagulants

Anticoagulants are substances employed to delay, suppress, or prevent clotting of blood. They are classified into 2 groups: the *in vitro* (outside the body) anticoagulants, and the in vivo (in the body) anticoagulants.

The commonly used *in vitro anticoagulants* include: EDTA, trisodium citrate, double oxalate, sodium fluoride, heparin, and ACD and CPD-A mixtures. The use of fluoride and heparin is limited to pH, blood glucose and gas analysis. The *in vivo* anticoagulants include: heparin and dicoumarol derivatives (warfarin, dicoumarin). Thus, heparin is both an *in vivo* and an *in vitro* anticoagulant.

4. Collection of venous blood

5. Collection of capillary blood

A. Selection of site

B. Apparatus

Keep the following equipment ready before venepuncture:

1. Disposable gloves. These should always be worn before venepuncture.
2. Sterile, disposable, one-time use, 10 ml syringe with side nozzle. Two 22-gauge needles with short bevels (the flattened puncturing points).
3. 10 ml test tubes, or vials, with or without anticoagulant.
4. Sterile gauze pieces moist with 70% alcohol/ methylated spirit.
5. Tourniquet. A 2–3 cm wide elastic bandage with Velcro strips to keep it securely in place. (It will be used to obstruct the venous return and make the veins prominent just before venepuncture). Alternately, a blood pressure cuff attached to its apparatus, or a ‘twisted‘ handkerchief can serve the purpose.

C. Procedure.

1. Seat the subject comfortably on a chair with an arm rest, or near a table. The subject, if nervous, may lie down on a bed. Reassure the subject by your approach and conversation.
2. Examine both arms in front of the elbows to locate a suitable vein. Ask your assistant to compress the upper arm with his hands to make the veins prominent. The antecubital (medial basilic) vein is embedded in subcutaneous fat and is usually sufficiently large to take a wide-bore needle. It also runs straight for about 3 cm, and is usually palpable—even in obese subjects. If the vein is neither visible nor palpable, try the other arm. (You should avoid superficial veins because they are notoriously slippery. Veins above the ankle or on the back of the hand may have to be used).
3. Once a suitable vein has been selected, support the subject's arm over the edge of the table. Wash your hands with soap and water, dry them on a sterile towel, and put on the gloves. Ask your assistant to open the syringe pack. Take out the syringe and attach the needle (it is attached/ detached with a little twist), with its bevel facing you.
4. Ask your assistant to apply the tourniquet about 2-3 cm above the elbow to obstruct the venous return. The subject may open and close her fist to increase the venous return and make the veins engorged (filled) with blood. If the vein is still not sufficiently prominent, a few 'slaps' with your fingers over the region may do so. Clean the skin over the selected vein with gauze and alcohol and allow it to dry. With the fingers of your left hand supporting and steadying the elbow from behind, stretch the skin over the vein downward with your left thumb placed about 4 cm below the vein. This traction fixes the vein and prevents its slipping when it is punctured.
5. With the piston pushed in, the side nozzle towards the subject's arm, and the bevel of the needle facing you, hold the syringe between your fingers and thumb of the right hand.
6. With the first finger placed near the butt of the needle, puncture the skin and push in the needle under the skin with a firm and smooth thrust, at an angle of 15-20° to the skin.
7. Slightly pull the plunger back with your thumb and little finger to produce a little negative pressure in the syringe. Advance the needle gently along the vein and puncture it from the side, a few mm ahead of the skin puncture. This prevents counterpuncture of the far wall of the vein and formation of a hematoma (local leakage of blood).
8. As the vein is punctured, all resistance will suddenly cease and blood will start to enter the syringe. With the needle still in the vein, and

The routine blood tests
1-Blood film
Is the study the cells of the blood is to examine fresh blood under the microscope in the form of a drop preparation and a thin blood film or smear made on a glass slide. In this experiment, the students will examine some features of blood cells and record their observations.

OBSERVATIONS

The red cells are non-nucleated, flat biconcave disks, round, oval or pear-shaped, thinner in the center and appear as colorless, or pale pink structures. (When stained with Leishman's stain, they appear dull orange-pink). Note if there is any rouleaux formation (cells lying on top of each other like a pile of coins) and the number of cells in a rouleaux. Observe if any leukocytes are seen, and their types if possible. Do they show any ameboid movement? Do you see any platelets—in clumps, or showing disintegration? After a time you may see fibrin threads when clotting starts.
2-Estimation of Hemoglobin(Hb g/dl) is the routine test, its part of complete hematological studies in all diseases of blood, especially types of anemia, leukemias, and chronic diseases such as TB, chronic infections, malignancies,….etc.
The average levels and their ranges:

- Males: 14.5 g/dl(13.5-18 g/dl)
- Females: 12.5 g/dl(11.5-16 g/dl)

What are the normal levels and ranges of **Hb at different ages?**
i. Newborns : 18–22 g/dl
ii. At 3 months : 14–16 g/dl
iii. 3 months–1 yr : 13–15 g/dl
iv. Adult males : 14.5 g/dl (13.5–17 g/dl)
v. Adult females : 12.5 g/dl (11.5–15.5 g/dl).
• There may be some decrease after age 60 years.

Sources and Degree of Error

False results with this method may be due to:
i. **Technical error.** It may be due to: not taking exactly 20 cmm blood, or not giving enough time for formation of acid hematin, or using an old comparator that has faded glass rods.
ii. **Personal error.** Generally, it is not difficult to match color but since it is a visual method, color matching may vary from person to person.

Q What are the common causes of anemia and how will you classify it ?

Normally, about 1% of RBCs are destroyed daily (cells present in about 50 ml of blood) and equal numbers (about 3 million/second) enter the circulation. Anemia may be caused when more cells are lost, or when less cells are produced, or both. Anemia will develop if:

i. Red cell production is normal but loss is increased.
ii. ii. Red cell production is decreased but loss remains normal.
iii. iii. Red cell production is decreased and loss is also increased.

Q Why is the Hb hemoglobin level high in the newborn?

The Hb may be as high as 20–22 g/dl at the time of birth due to the high red cell count (> 6 million/ mm3). The newborn has been living in state of relative hypoxia, which is a very potent stimulus for the secretion of EP. As age advances the Hb levels decrease, and adult levels are reached in a few years.

Q Compared to males, why are the Hb levels lower in females?

All other factors (age, body mass, etc.) being equal, the levels are lower in healthy females not because of menstrual loss of blood (20–30 ml). It is the estrogens in the females which have an inhibitory effect on the secretion of erythropoietin (EP) which is the main stimulant of red cell production. Also, the androgens (mainly testosterone) have a stimulatory effect on EP secretion. Both these factors tend to keep the red cell count higher in males.

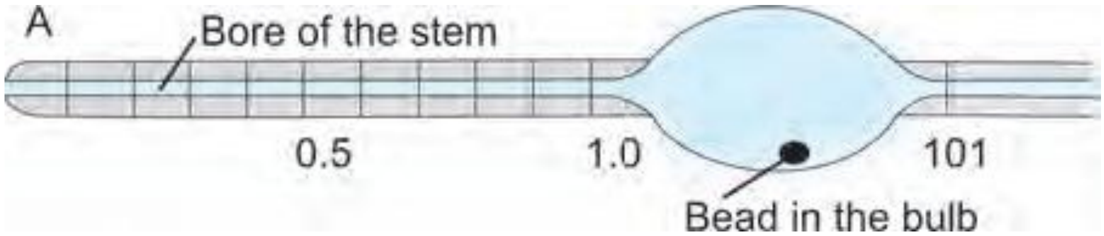
Q Mention the common causes of increased and decreased Hb reading?

3- The red blood cells count (RBCs)

Normal red cell count: the average cell counts and their ranges are:

- Males = 5.0 million/mm^3(4.75-6.0 million/mm^3)
- Females = 4.5 million/mm^3(4.0-5.5 million/mm^3)

RBC count, if done carefully and as part of full blood count (FBC), is required for the calculation of absolute corpuscular values and indices in anemia.



Calculation of red cell count

undiluted blood will be = x × 50 × 200

for example the RBCs count =480

∴ Number of cells in 1 mm3 of undiluted blood will be

= 480 × 50 × 200

= 480 × 10,000

= 4800000, i.e., 4.8 million/mm3.

Q- Which physiological condition causes a **decrease in RBC count?**