Blood collection and Determination of Hematocrit (Hct)(Packed Cell Volume; PCV)

Physiology Lab-1

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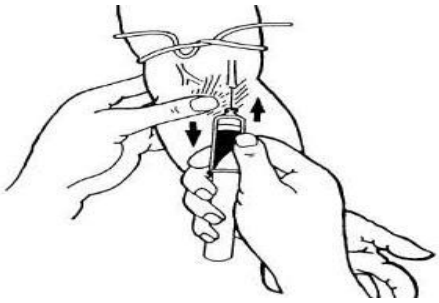
Collecting blood samples

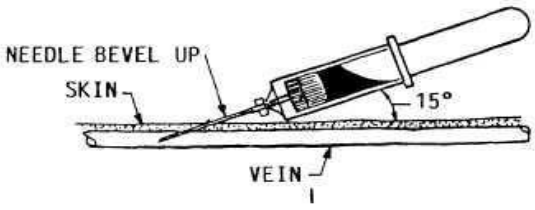
Is crucial to the understanding, prevention, and treatment of disease. However, from the patient's perspective, it can also be painful, unnerving, frightening, and inconvenient.

Three popular methods of blood collection are

1. Arterial punctures sampling: This method is not common in obtaining blood samples, used special in an arterial-blood gas (ABG) test measures the amounts of arterial gases, such as oxygen and carbon dioxide. An ABG test requires that a small volume of blood be drawn from the radial artery with a syringe and a thin needle.

2. **Venipuncture sampling:** Venipuncture is the most common way to collect blood from adult patients, testes needs large amount of blood. Collection takes place from a superficial vein in the upper limb, (generally the median cubital vein) this vein is close to the skin and does not have many large nerves positioned close by. This reduces pain and discomfort for the patient.





Materials that will used in this methods:

EDTA tubes, Needle and syringe, Tourniquet, Skin antiseptic solution: 70% isopropyl alcohol, Gauze pads, Adhesive bandage.

Steps of collection:

1. Apply tourniquet on the upper arm about 5 inches above cubital vein.
2. Clean the arm (antecubital region) using 70% alcohol to the outside using anti-clock wise direction. 3. Don't retouch the area because it will contaminate the area.
4. Remove cap from needle, bevel edge of needle facing up.
5. Inject it at 15-30 degrees parallel into the median cubital vein to draw blood.
6. When blood starts to flow, ask patient to open his/her hand.
7. Release the tourniquet before withdrawing the needle. 8. Remove the needle and place cotton gauze on the site of injection to stop bleeding.
9. Fill the tube 3\4 with blood according to the anticoagulant concentration and the ratio mandated.

10. Finally mix the blood in the tube by making number 8 symbol in the air while holding the test tube.

3. Capillary blood collection: is the preferred method of blood specimen collection for newborns and infants. Capillary blood collection via (heel stick for infants less than one year of age). For children (older than one year, capillary blood collection via finger stick should be considered.

a) **Finger stick sampling**: Finger stick or finger prick sampling involves taking a very small amount of blood from the patient, usually from the end of a finger. The index finger is often hard-skinned potentially more sensitive to pain due additional nerve ending. The thumb also may be hard- skinned and has a pulse, indicating arterial presence, and therefore, should be avoided. The distance between the skin surface and the bone in the fifth finger also makes it unsuitable for puncture. The side and tip of the finger should be avoided, as the tissue is about half as thick as the central portion of the fingertip.

Hematocrit:

- ✓ Hematocrit is defined The percentage by volume of packed red blood cells in a given sample of blood aftercentrifugation.
- ✓ The hematocrit may also be referred to as Packed Cell Volume (PCV) or erythrocyte volume fraction (EVF).

Objective

- To pack the RBC using the microhematochrate centrifuge force.
- Forcing all red cell below and plasma above, by centrifugal force.

Relevance

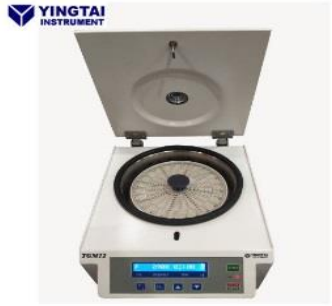
Measurement of hematocrit (Hct) or packed cell volume (PCV) is the most accurate and simplest of alltests in clinical hematology for detecting the presence and degree of anemia or polycythemia. In comparison, hemoglobin estimation is less accurate, and RBC count far less accurate.

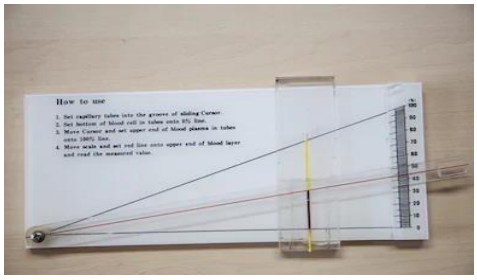
Methods

- Microhaematocrit
- Electronic cell counting

Material and instruments

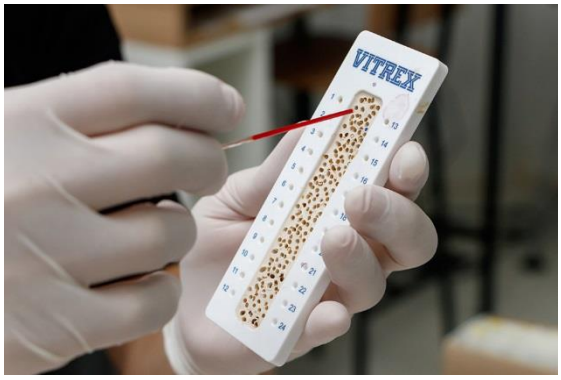
1. Microhaematocrit tube (capillary tube) 75mm in length and 1mm in diameter, which contains heparin, and show a red ring at the end of the tube.
2. Microhaematocrit centrifuge device.
3. Plastic seal: to seal one end of Microhaematocrit capillary tube.
4. Microhaematocrit reader.

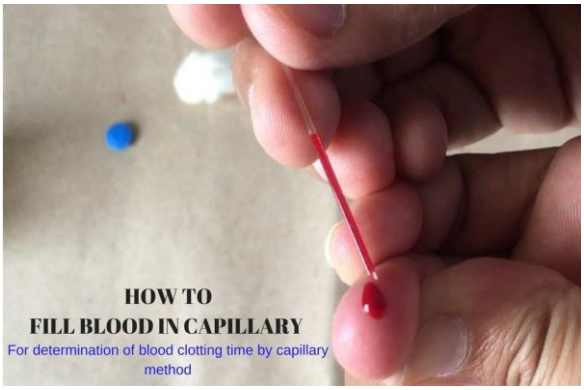


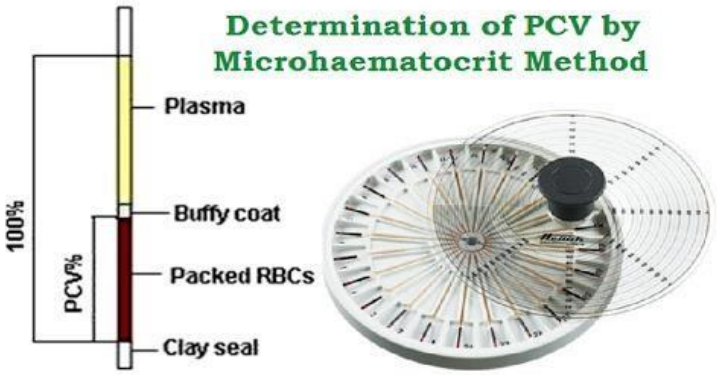


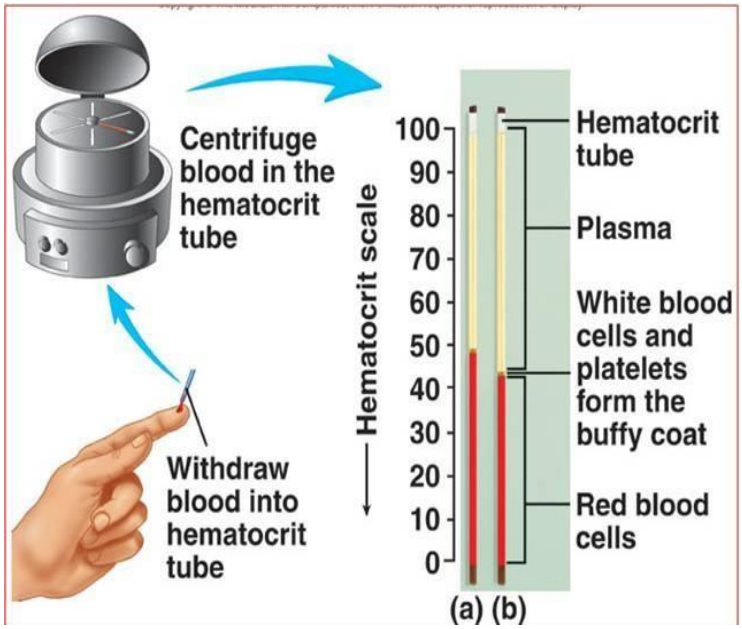
Procedure:

1. Clean your finger with 70% alcohol and let dry.
2. Prick finger with lancet, near the tip but not too close to the nail. Prick so that blood flows freely. Try squeezing up from your wristif blood does not flow after pricking finger.
3. Place the tip of a capillary tube onto a drop of blood on your finger.
4. Call your instructor to seal the tube.
5. The instructor will spin the tubes in a centrifuge (5 minutes at10000 rpm).
6. Using a special reading device(since the capillary tube is notgraduated).



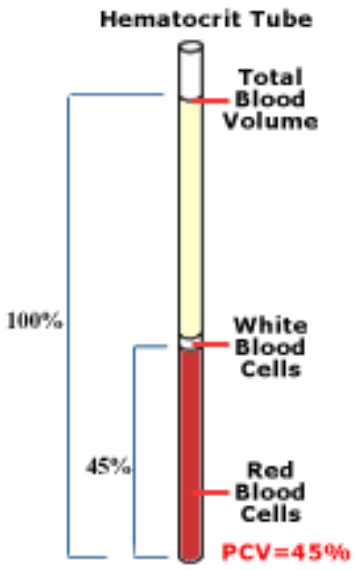


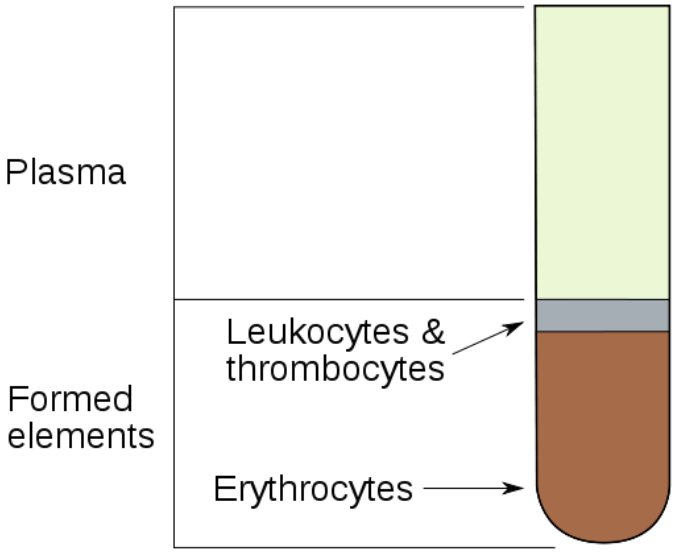




Observations and Results:

- ☒ Note that the blood has been separated into 3 layers:
- ☒ A tall upper layer of clear plasma—amber slightly yellow-colored. It should not be pink or red, which would indicate hemolysis of red cells in the sample or within the body in hemolytic diseases.
- ☒ A greyish-white, thin layer (about 1 mm thick) the so- called "buffy layer", consisting of platelets (thrombocytes)above and leukocytes below it.
- ☒ A tall bottom layer of red cells.





Results

The percentage of the volume of blood occupied by the red cells constitutes hematocrit or packed cell volume.

Hct % = {Height of RBCs (mm) / Height of RBCs and plasma (mm)} ×100

For example, if the height of packed red cells is 45 mm, then

= 45/ 100 × 100 = 45 percent.

- It also means that out of 100 volumes (or parts) of blood 45 volumes (or parts) are red cells and 55 volumes (or parts) are plasma. Thus, out of 1 liter of blood,450 ml are red cells and 550 ml are plasma.

Normal values.

The normal values of PCV vary according to the age and sexof the individuals. The normal ranges are:

Males: 40 %–54 %

Females: 36 %–47 %

Newborns: 55-68 %

Clinical implications:

PCV is affected by the shape, & the number of the RBCs & the plasma volume.

High PCV either indicates either increase in number of circulating RBCs or decrease in plasma volume seen in choleradue to loss of water in the stool

A low PCV indicates either decrease in RBC or increase in plasma volume

Clinical implications:

A lower than normal hematocrit may indicate:

1) An insufficient supply of healthy red blood cells (anemia)
2) A large number of white blood cells — usually a very small portion of your blood — due to long-term illness, infection, leukemia, lymphoma or other disorders of white blood cells.
3) Acute kidney disease (lower Erythropoietin production lead to Less RBCs production by the bone marrow).

4) Pregnancy may lead to women having additional fluid in blood. This could potentially lead to a small drop in hematocrit levels

Clinical implications:

A higher than normal hematocrit may indicate:

1) Abnormal increase in red blood cells (erythrocytosis)
2) A disorder, such as polycythemia vera that causes yourbody to produce too many red blood cells (in polycythemia it may rise to as high as 7%).
3) At higher altitudes, there is a lower oxygen supply in theair and thus hematocrit levels may increase over time.
4) Low blood oxygen levels (hypoxia)
5) Lung or heart disease — if the body senses low oxygenlevels, it will make more red blood cells in an effort to increase the amount of oxygen in the blood
6) Dehydration.
7) Burn(due to loss of plasma)

Sources of error and comments

- An increased amount of anti-coagulant decreases the
- Hct reading as a result of erythrocyte shrinking.
- Improper sealing of the capillary tube causes a decreased Hct reading as a result of loss of blood duringcentrifugation. a higher number of erythrocytes are lostin relation to the plasma.
- The microhematocrit centrifuge should never be forced to stop by applying pressure to the metal cover plate. This will cause the RBClayer to “sling” forward and results in a falsely elevated value.

Sources of error and comments

- The buffy coat of the specimen should not be included in the Hct reading, because its inclusion falsely elevatesthe result.
- A decrease or increase in the readings may be seen if themicrohematocrit reader is not used properly.
- If too much time elapses between when the centrifuge stops and the capillary tube is removed, the red cells canbegin to settle out and cause a false reading of the hematocrit.

Date----------
Group--------

Names of the students**:**

Name of the experiment:

Aim of the experiment:

Materials

Procedure:

1. Clean your finger with 70% alcohol and let dry.
2. Prick finger with lancet, near the tip but not too close to the nail. Prick so that blood flows freely. Try squeezing up from your wrist if blood does not flow after pricking finger.
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Result:

Discussion: