Clinical pathology: Measurement of haemoglobin: 3-4th practical lecture

Indication: Evaluation of erythron, diagnosing and typing anaemia. There are many laboratory methods to measure Hb concentration manually, the available but not the best is the Sahli's method (acid hematin method).

Materials:

1-Sahli's haemoglobinometer, with standard color box graduated tube (it has two graduations, a percentage and gram/ deciliter), glass rod, and 20 µl pipette. Haemolysing agent, (0.1N) hydrochloric acid that will react with the freed haemoglobin forming acid hematin (brown in color).

2- Fresh blood sample.

Procedure:.

a- Using pasteure pipette put diluting fluid in the graduated tube to the mark (10) of the percentage graduation.Mix blood gently.

b- Using sahli's pipette draw 20 µl of blood ,wipe excess of blood from outside of the pipette.

c- Introduce the pipette to the bottom of the tube expel the blood, mix by sucking and expelling HCL solution 2-3 times until all rests of blood is removed from the pipette.

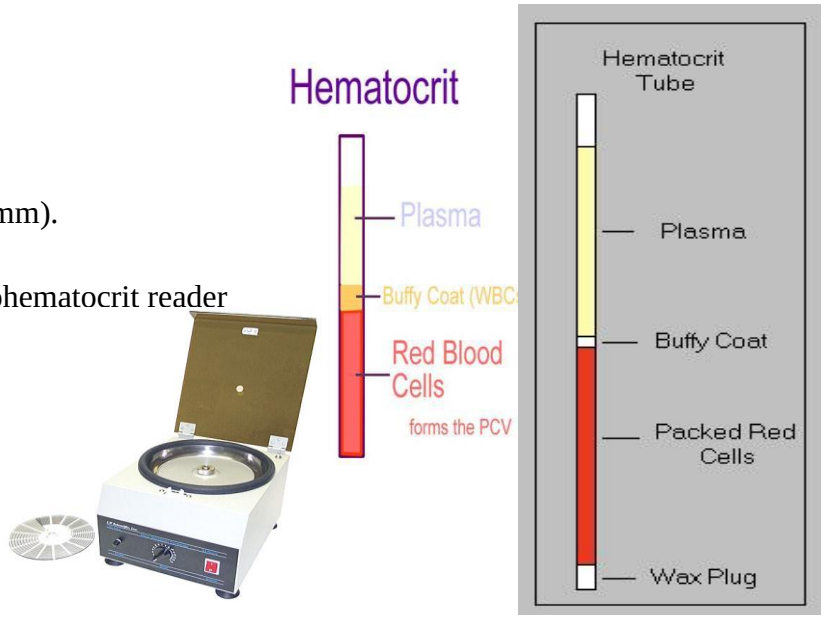
d- Mix by a rotatory movement using glass rode, leave the mixture for 2-3 minutes to achieve complete haemolysis.

e- Add distal water drop by drop, mix well by the glass rode and compare with the standard color in the box, until colors are matched correctly.

f- Haemoglobin concentration is measured directly by observing the figure which the meniscus has reached in the g/dl graduation.

PACKED CELL VOLUME (PCV) OR MICROHAEMATOCRIT VALUE (HCT): It is a measurement of the relative percentage of red blood cells in a particular volume of blood..

Indication: Evaluation of erythron, diagnosis and typing of anaemia, diagnosis of polycythemia.



MATERIALS:

1- Fresh blood.
2-Two capillary tubes (1x70 mm).
3- PCV centrifuge.
4-PCV-reading scale or microhematocrit reader
5. Clay.

Method:

a- Mix blood gently.

b- Fill the two capillary tubes with blood till 2/3 or 2/4 of its length, wipe outside of the capillary tubes from blood.

C- Seal one end with clay to about one centimeter.

d- Put them in the radial groove of pcv centrifuge exactly opposite to each other with the sealed end to the outside.

e- Centrifuge for 5 minuets at $12x10^3$ RPM .Blood will be separated into three layers as shown in the figure.

f- Measure the percentage of rbcs or the pcv using the reader.

Note : Advantages that you may get by examining a centrifuged capillary tube:

- **Dark yellow plasma indicates jaundice.**
- **Whitish cloudy plasma indicates lipaemia as in diabetic dogs.**
- **Pinkish plasma indicates a haemolytic disease.**
- **Thick buffy coat layer indicates either leukocytosis or leukemia.**
- **Pinkish–colored buffy coat indicates responsive anemia, immature RBCs are found in this layer.**

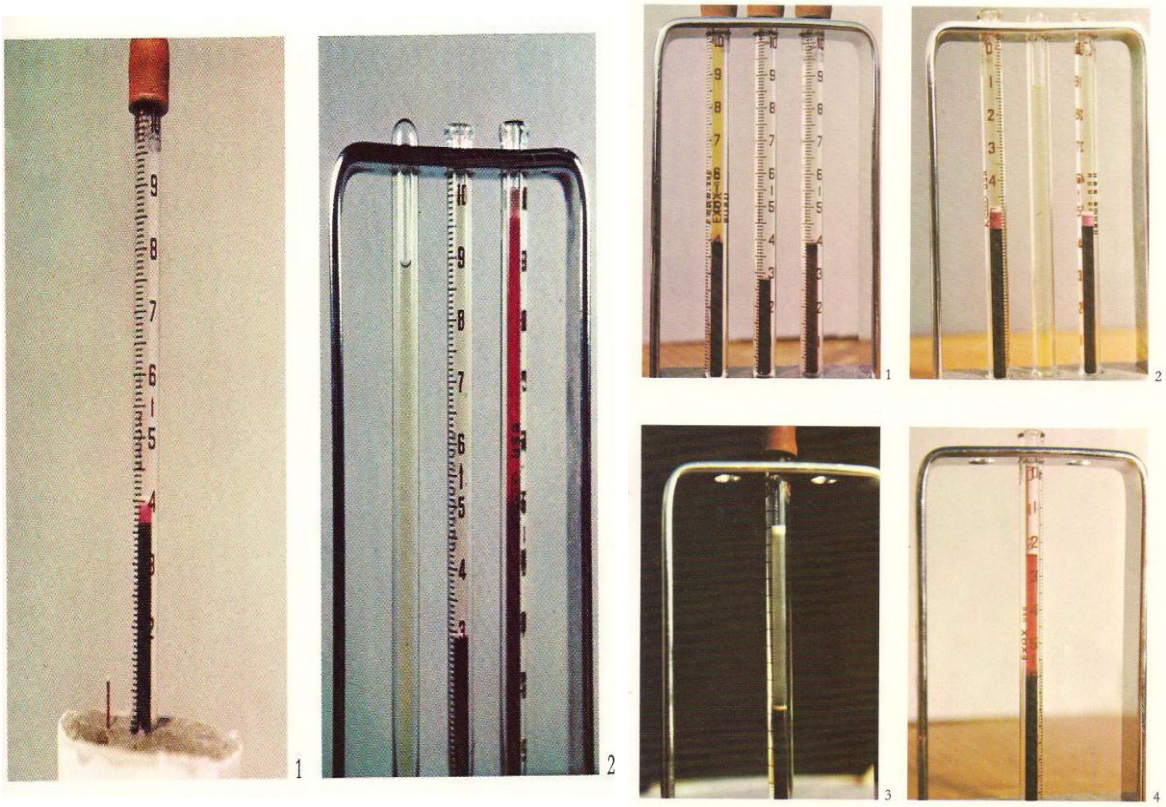


Table (1): Normal Hb, PCV & RBCs values Some farm animals .

Animal species	Hb g/dl	Pcv%	RBCsx$10^{6/\mu l}$
Cow	8-15	24-46	5-10
Horse	11-19	32-52	6.5-12.5
Dog	12-18	37 55	5.5-8.5
Sheep	9-15	27-45	9-15

Note : The HCT is calculated from the RBC count and the mean corpuscular volume (MCV), using the following equation:

Hematocrit (%) = (RBC x MCV)/10