**Hemoglobin Determination**

Hemoglobin is the major constituent of the red cell cytoplasm, accounting for approximately 90% of the dry weight of the mature cell. It is comprised of heme and globin Various methods are available for estimation of hemoglobin in the laboratory. Function of Hemoglobin .Heme has the ability to bind oxygen reversibly and carry it to tissues. It also facilitates the exchange of carbon dioxide between the lungs and tissues. Thus, hemoglobin functions as the primary medium of exchange of oxygen and carbon dioxide. Many methods are available

1. Methods based on development of color. These are

Sahli's or acid hematin method , Cyanmethemoglobin method , Oxyhemoglobin method, Alkaline hematin method.

2. Measurement of oxygen combining capacity

3. Measurement of iron content

Sahli's or acid hematin method

Objective: To determine the amount of hemoglobin present in 100 ml of blood of a given sample. Significance

a. It serves as an index of blood condition of the animal.

b. If the hemoglobin [Hb] content falls below the normal levels, it indicates anemia, or pregnancy (physiological).

c. If it increases to the normal value, it indicates polycythemia, decrease in O2 supply, heart disease, emphysema, dehydration etc.
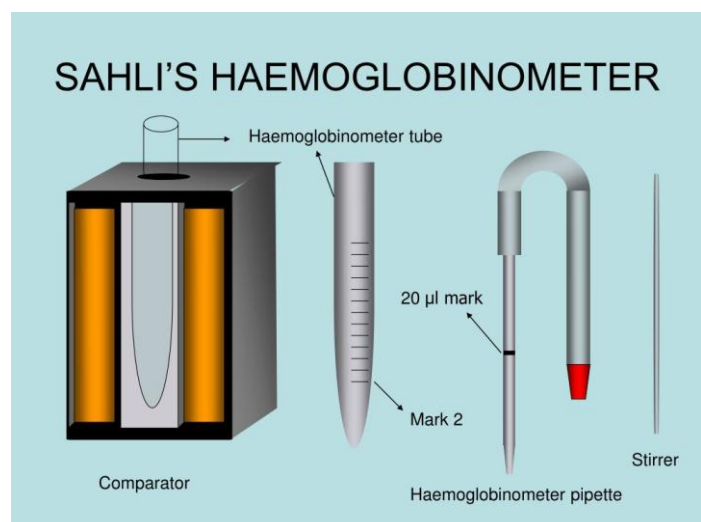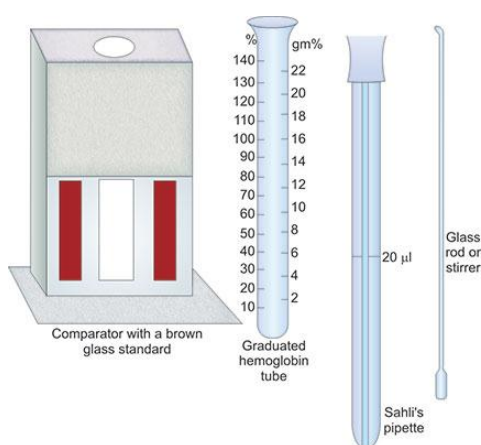
Procedure

• Take 0.1N HCl (1%) into central graduated tube up to mark 2.

• Suck the blood exactly up to mark 20 (20 μl) with the help of sahlis pipette.

• Transfer the blood from pipette to central graduated tube of the hemometer.

• Mix it well with the help of stirrer or rod and allow it to react for two minute.

• Make up with distilled water by adding drop by drop until the color matches with the Standard comparator tube and mix well.

• When the color matches take out and record the values on the side as gm/100ml and or in percentage.

• Repeat 5 to 6 times and take the average value

Normal value: Bovine 8 - 15gm/dl (11)   Equine 11 -19gm/dl (14)

Ovine 9 – 15gm/dl (11.5)   Feline 8 – 15gm/dl (12)

Caprine: 8-14gm/dl (11)   Canine 12 – 18gm/dl (15)

Human Adult males :- 14.0 - 17.5 gm/dl.   Adult female:- 12.3 - 15.3gm/dl.

Errors-:

1. Technique of collecting blood sample.

2. Manual error: In labelling, in method, in visual comparision.

3. Instrumental: Comparator glass standards may fade with time, so dilution is more in the specimen hence higher values of Hb are obtained.Calibration errors.Improper light can lead to variation in result.

4. All forms of Hb are not measured as only Hb and HbO2 can be converted to acid hematin. So it does not give the true value of hemoglobin.

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Hematocrit Determination (PCV)

Hematocrit or packed cell volume (Hct/PCV) is the volume occupied by red cells after blood is centrifuged at a high speed.Hematocrit is measured by two methods:

1. Microhematocrit method (not used now).

2. Automated method .

• Reduced Hematocrit is seen in anemia.

• Increased hematocrit is seen in polycythemia.

- Buffy coat; white to gray layer above PCV. It will give number of WBC (0.5mm to1.5mm).Leukopenia or leukocytosis.

- Plasma content: usually about 55%, Yellowish in color. Degree of yellowness indicates icterus (jaundice).

Aim: To determine the hematocrit value for a given blood sample.

Principle: Blood compartment is separated into three parts using capillary tube in a hematocrit centrifuge.

Method: Wintrobe hematocrite method

Requirements: Hematocrit tube, hematocrite centrifuge, hematocrit reader and sealer.

Procedure

• The blood is filled in to a micro hematocrit tube (3/4th) and seals it with sealer.

• Centrifuge the filled hematocrit tube in a hematocrite centrifuge at 2000 rpm for 4-5 minutes.

• Read the value (the tube) with hematocrit reader and record the result.

Normal value

• Bovine 37 - 55 % (45%)

• Ovine 24 - 50 % (38%)

• Caprine 24 - 50 % (40%)

• Equine 32 - 55 % (42%)

• Swine 32 - 50 % (42%)

Precautions
1. PCV increases with storage so it must be measured within 6 hrs of sample collection.
1. Capillary tubes must be of defined specifications.
2. Centrifuges must be checked at regular intervals for speed and accuracy.

OBSERVATION
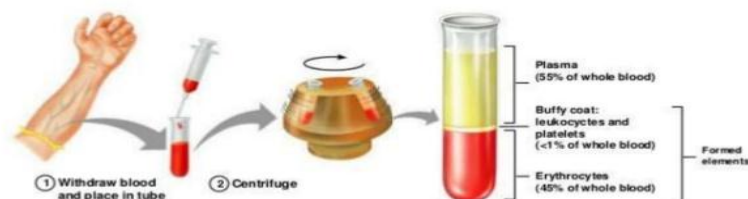
- **Hematocrit**
  - **Males: 44%(38—50%)**
  - **Females: 42% (36—45%)**