Total Count of RBC

Erythrocytes are counted via hemocytometer

which is consist of :

a. Thoma blood cell diluting pipettes with plastic mouthpiece
b. Double cell counting chamber with Neubauer ruling central

Procedure of erythrocytes count :

1. Blood should be carefully drawn to the 0.5 mark of the pipette .
2. An isotonic solution such as normal saline or Hayem's solution drawn to the mark 101 and well mixed .
3. Discharged onto the hemocytometer counting chamber and allowed to settle for several minutes. Before the areas in the chamber are counted, the slide should be examined under low power to check distribution of cell in the ruled area .
4. Count RBCs in five squares in the center of the counting chamber, then multiplied by 10.000 .This value represents the total number of erythrocytes per microliter .

Note: Some difficulty may be encountered in counting the same cell twice. One method of avoiding duplication is to count only those cells that touch the lower and right boundaries.

Hemoglobin Determination

Methods for hemoglobin determination are many and varied such as Acid haematin method(sahli method) and cyanmethaemoglobin method. The manual methods for determining blood hemoglobin is the acid hematin method that use Sahli hemoglobinometer. The blood is mixed with dilute hydro-chloric acid which hemolyzes the red cells, disrupting the integrity of the red cells' membrane and causing the release of hemoglobin, which is converted to a brownish-colored solution of acid hematin. The acid hematin solution is then compared with a color standard.

1. Add N/10 hydrochloric acid to the 20 mark on the graduated tube.
2. Fill the pipette with the blood to the mark 20 µ, and expelling it into the acid solution, followed by rinsing out the pipette to ensure thorough mixing. HCL convert the haemoglobin to haematin which has a brown color.
3. The tube and contents should be removed from bright light and left 5 minutes until acid hematin has developed.
4. Dilute the contents with distilled water until the color matches that of the glass standard.

Normal value: Bovine 8 - 15gm/dl Equine 11 -19gm/dl
Ovine 9 – 15gm/dl Feline 8 – 15gm/dl
Caprine: 8-14gm/dl Canine 12 – 18gm/dl

Hematocrit Determination (PCV)

The packed cell volume (PCV), or hematocrit (HCT) represents the proportion of blood composed of red blood cells, expressed as % (vol/vol). It is the quickest and most accurate measure of the red cell component of blood. Microhematocrit is the suitable method for routine use.

The procedure as follows:

1. Thoroughly mix the blood samples and fill capillary tubes to approximately 3 quarters of their length with the samples.
2. Wipe excess blood from the outside of each tube, then seal the opposite end by plugging with a plastic sealing compound.
3. Place the capillary tubes in the grooves of the base plate of the microhematocrit centrifuge with the sealed end pointing outwards. Note the position and identity of each tube
4. Firmly secure the inner lid of the centrifuge, then close the outer lid. Centrifuge for five minutes at 12000 rpm.
5. when the centrifuge has stopped rotating open the lid remove the safety plate and take out the tubes.
6. Read the PCV by a special reader as follows:

a. Place the bottom of RBC column (which is just above the sealed end) exactly on the base line of the reader (0).
b. Move the reader until the top of the plasma layer exactly meets upper line.
c. Observe a line on the reader which passes across the top of the RBC layer.
d. Read the PCV from the scale at the point where this line meets it.

Normal value

- Bovine 24-46 %
- Ovine 27-45 %
- Caprine 22-38 %
- Equine 32-53 %

Determination of Erythrocyte Sedimentation Rate (ESR)

It is a rate of sedimentation of erythrocytes in column of anticoagulated blood at certain time. It is measured in (mm). When blood containing anticoagulant is allowed to stand in a perpendicular tube, the erythrocytes sink because they are heavier than the plasma in which they are suspended. The speed with which erythrocytes fall in the blood of normal animals is relatively slow, but in animals with inflammatory diseases such as(pleura, pericardium and peritoneum), And in infection disease , inflammation skin, and traumatic injury including surgery the speed is increased. Retarded ESR in allergic disease, congestive heart failure, polycythemia and sickle cell anaemia. This alteration in suspension stability probably results from changes that occur in the physiochemical properties of the erythrocyte surfaces and the plasma.

1. Mix the blood well and draw the sample into clean dry Westergren tube that has a length of about 30 cm and a bore of 2.5 mm
2. Place the tube into the stand, taking care that the base is firmly positioned on the base pad to prevent leakage.
3. Adjust the rack so that the tube rests in an exactly vertical position.
4. Leave undisturbed for 20 minutes in horses, 1 hours in dog and cats, 24 hours in ruminants. It is conventional to set up sedimentation rates at room temperature (18 - 25^{o}C).
5. At the end of the time, read the height of clear plasma above the upper margin of the column of sedimenting cells to the nearest millimeter.