Leukocytes count

1. Total Leukocytes count (TLC):

Hemocytometer is used for enumeration of total leukocytes.

a. Carefully blood drawn to the 0.5 mark of the pipette.
b. The diluting fluid (Turck's solution) is then drawn to the mark 11 and well mixed.
c. Discharged onto the hemocytometer counting chamber as done in erythrocytes count.
d. The total number of WBCs in four squares of larger ruled area in the corner of the counting chamber is determined and multiplied by 50. This value represents the total number of leukocytes per microliter.

2. Differential Leukocytes count (DLC):

Differential leukocytes are counted by blood film. The blood film should be made from fresh blood containing no anticoagulants (as soon as possible after collection of the blood) ; otherwise, best results are obtained if EDTA is used as the anticoagulants. Films of blood may be prepared either on a microscope slide or coverslip.

Microscope slide method:

1. Select good quality, clean, grease-fresh slides.
2. Place a slide on a flat surface, or held by edges between the forefinger and thumb.
3. Place small drop of well-mixed blood near one end of the slide by using of applicator stick or capillary tube.
4. Immediately after placing blood on the slide, place second slide "spreader" in front of the drop of blood at an angle of approximately 30˚ and pull it back until it comes to contact with the drop of blood, and the pause until the blood spreads along the edge of the spreader
5. After the blood spreads along the edge of the spreader, push the spreader forward smoothly and quickly at an angle about 30˚. The greater the angle the thicker and shorter blood smear, and the smaller the angle the thinner and longer smear.
6. Dry blood film quickly by waving it in the air.
7. Identify the blood film by writing in the date and client's name, or a reference number, along the end of smear with a pencil or the edge of the spreader slide.
8. Whenever possible fix and stain blood films immediately they are prepared, otherwise fix them in absolute methanol and then store them in a clean box until they can be stained.

Coverslip method:

1. Square coverslip 22 by 22 mm of No. 1 thinness are used.
2. Hold a clean, dry coverslip by its edges in one hand.
3. Place a small drop of blood on the center of the coverslip.
4. Place a second clean, dry coverslip diagonally on the first, forming an eight-pointed star; the blood will immediately spread.

5. Grasp the top coverslip by its corners and using a smooth motion slide the two apart , then wave the coverslip in the air to enhance drying.
6. The coverslips should be identified by placing it in a small box with the name of the owner or number of the case on it.

Staining the blood smear

1. Leishman's staining method
a. Place the slide on a staining rack.
b. Cover the smear completely with leishman's stain and leave it to act for 1-2 min. this fix smear.
c. Add to the slide a volume of buffered distilled water which is approximately twice that of the stain already present. The gently rocking the staining rack evenly mix the buffered distilled water and stain.
d. Allow the mixture to act for 10- 15 min.
e. Wash off the mixture from the slide with buffered distilled water, take care to avoid any precipitate of stain remaining. Flood the slide with buffered distilled water and allow it to remain on the slide for approximately 1 min. until the smear has a pink tinge.
f. Pour off the water and dry the slide.

2. Giemsa stain
a. Place dried blood smear in staining jar containing absolute methyle alcohol for 3 – 5 min.
b. Drain off the alcohol and allow the slide to dry.
c. Transfer slide to staining jar containing Giemsa stain and allow to stain for 15 to 60 min.
d. Wash slide thoroughly with buffered distilled water .

Method of differential Leukocytes count:
1. The examination is made with oil-immersion objective.
2. Count and differentiate 100 leukocytes
3. The individual cells can be tabulated in columns on a prepared sheet of paper, or a blood cell counter .

White blood cell reference values

	Cattle	Sheep	Goat	Horse
WBC (per/µL)	4000-12 000	4000-12 000	4000-13 000	5400-14 300
Neutrophils (per/µL)	600-4000	700-6000	1200-7200	2300-8500
Lymphocytes (per/µL)	2500-7500	2000-9000	2000-9000	1500-7700
Monocytes (per/µL)	25-800	0-750	0-550	0-1000
Eosinophils (per/µL)	0-2400	0-1000	0-650	0-1000

Hemostasis and Coagulation of Blood:

Whole blood coagulating time

The whole blood coagulating time (Clotting time) can be determined by several techniques. A simple method is the capillary tube method:

1. The skin is punctured, the first drop of blood is wiped away, and the capillary tube is filled with blood noting the time when blood first appears in the capillary tube.
2. Holding the tube between the thumb and index finger of both hands.
3. Gently break off small pieces every 30 seconds until a strand of fibrin is seen extending across the gap between the two broken ends of the tube.
4. The interval between the appearance of blood and the appearance of a fibrin strand is the clotting time.

Normal values coagulation time

Bovine	3-5
Ovine	1-6
Caprine	2.5-11.5
Equine	3-15
Canine	3-4
Feline	5

Bleeding time

Determination of bleeding time is a simple and sometimes useful tool for evaluation the efficiency of the capillary-platelet aspect of hemostasis. The technique as follows:

1. With the animal suitably restrained, or even anesthetized, carefully clip an area of skin where there are comparatively few hairs. Wash the area thoroughly with soap and water, and dry it.
2. Using a sterile disposable lancet, swiftly make 2 small puncture wounds in the skin a short distance apart avoiding any major blood vessels.
3. Start the stop-watch. At 30 seconds intervals, gently touch the drop of blood on each wound with a piece of filter paper. Take care not to touch the edge of the wound because this may dislodge the platelet plug.
4. When no spot of blood appears on the filter paper, read the time.

Normal values of bleeding time

In most domestic animals 1- 5 min.

Hematology reference values

	Cattle	Sheep	Goat	Horses
Hemoglobin (g/dL)	8.0-15.0 9.	0-15.0	8.0-12.0	11 .0-19.0
Hematocrit (%)	24-46	27-45	22-38	32-53
RBC ($x10^6$/µL)	5.0-10.0	9.0-15.0	8.0-18.0	6. 8-12.9
MCV (£1)	40-60	28-40	16-25	37-59
MCH (pg)	11 .0-17.0	8.0-12.0	5.2-8.0	12.3-19.7
MCHC (g/dL)	30 -36	31.0-34.0	30.0-36.0	31.0-38.6
WBC (per/µL)	4000-12 000	4000-12 000	4000-13 000	5400-14 300
Neutrophils (per/µL)	600-4000	700-6000	1200-7200	2300-8500
Lymphocytes (per/µL)	2500-7500	2000-9000	2000-9000	1500-7700
Monocytes (per/µL)	25-800	0-750	0-550	0-1000
Eosinophils (per/µL)	0-2400	0-1000	0-650	0-1000