HAEMATOLOGY

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Complete blood cell count (CBC) means examination of blood and plasma for packed cell volume (PCV), haemoglobin percentage (Hb. %), total leucocyte count (TLC), total erythrocyte count (TEC), differential leucocyte count (DLC), erythrocyte sedimentation rate (ESR) etc.

Haematological tests for convenience can be categorized as follows:

(a) Erythrocyte related tests	-Haemoglobin estimation
	-Packed cell volume
	-Total erythrocyte count
	- Morphology
(b) Leukocyte related tests	- Total leukocyte count
	- Differential leukocyte count
	- Morphology
(c) General/non-specific	-Erythrocyte sedimentation rate
	- Platelet/ Thrombocyte count
	- Erythrocytic indices

Haemogram: It is the blood picture obtained from performing complete blood count of a patient.

COLLECTION OF BLOOD

Venous blood is needed to be collected for different haematological examinations.

General consideration:

1. Blood should be collected when the animal is at rest and without undue excitement to avoid alteration in the haemogram and some blood chemistry values. The anticoagulant selected should not alter the size of RBC, do not cause hemolysis, minimize platelet aggregation, minimize disruption of WBC and can be readily soluble in water. Best recommended anticoagulant is K_2 EDTA.

2. Serum rather than plasma is preferred for most chemical analysis as anticoagulants may interfere.

Amount of blood: It depends on test required and the methods used.

i. For aid in diagnosis of diseases smallest quantity necessary for test be collected. Do not collect blood more than 0.5 ml/ kg b. wt.
ii. For clinical chemical profiles a minimum of 2.5 ml of serum is needed.

Requirements:

Hypodermic needles (gauge 16 & 26), Syringes (2-5 ml), Collection vials/tubes containing anticoagulants.

Procedure for collection of blood:

i. Clip the hairs from the site of blood collection.
ii. Apply antiseptic (ethanol/tincture of iodine) over the site.
iii. Raise the vein by pressure.
iv. Insert the needle into the vein and collect the blood in dry syringe for required quantity of blood (5 ml in case of large animals and 2 ml in case of small animals).
v. Disengage the needle and transfer blood from syringe to anticoagulant containing vial. Mix the blood using rotational movements in the vials.

Precautions:

i. Use dry syringe and needles.
ii. Never shake the blood which will cause rupture of blood cells.
iii. Remove needle before filling collection tubes.
*If not, it will cause hemolysis and interfere with test like serum lipase, bilirubin, icterus index, urea nitrogen, enzymes, pH etc.
iv. Do not fill the blood collecting vials up to top rather allow adequate space for mixing.
v. Blood smears should be made from fresh blood preferably or with 15 minutes of collection of blood.
vi. Fast the animal before collection as lipemia may result.
vii. The whole blood should not be held for longer time as will be deteriorated.
viii. Do not take too much time in obtaining blood as clot will result.
ix. Agitate gently and properly the blood to mix with the anticoagulant.

ANTICOAGULANTS:

1. EDTA (K or Na salts of ethylenediamine tetraacetic acid): 10-20 mg/ml of blood or 1 ml of 1% solution dried at room temperature. Acts by forming insoluble Ca salts. It is recommended for routine haematology. Excess amount causes cell shrinkage.
2. Heparin: 1-2 mg/10ml of blood. Antithrombin and antithromboplastin action. Not suitable for smears.
3. Ammonium and Potassium oxalate (Heller's and Paul's double oxalate). 20mg/10ml of blood. Acts by forming insoluble Ca salts. Can be used for most haematological procedures.
4. Other commonly used anticoagulants are-Sodium citrate, Potassium oxalate, Sodium oxalate and ACD.

BLOOD SMEAR

A well stained blood smear by a knowledgeable observer can provide more valuable information than any other single laboratory test there are two methods for preparing blood smear :-

1. Coverslip method – Distribution of cells is best but difficult to handle and clean.
2. **Slide method** – Slide blood smear can easily be made and gives adequate results and routinely followed. The method is described in detail:

Requirements:

- Fresh blood/sample collected in anticoagulant preferably with EDTA.
- Clean, dry slides with smooth edges.
- Stains-Giemsa, Wrights, Leishman.
- Methanol/ethyl alcohol/ formaldehyde.
- Applicator stick/capillary tube.
- Mechanical cell counter.

Procedure:

- Mix the blood sample by rotation
- Take a small drop of blood on one end of a slide on dry grease free smooth edged slide using applicator stick/capillary tube.
- Bring spreader slide at 30˚ angle against the surface of first slide near blood drop and ensure blood spread to 2/3rd of slide, the spreader is gently pushed with steady and even motion to

draw blood smear.

- The smear is dried by waving in air and avoid blowing.
- Fix the smear with methanol (not required while using Romanowsky stains i.e. Wrights & Leishman) for 3 minutes.
- Stains the slide with Giemsa/Wrights stain.

Characteristic of a good blood smear:

- It should be thick at one end thin and feathered at the other.
- The edge of film should be at least 2 mm from the slide edge.
- The smear should have a smooth appearance and should be free from holes. Presence of holes indicates grease on slide/spreader.
-It should have straight borders and rainbow like appearance when seen against light.
- There should not be any jerks in it.
- It should cover 2/3 area of slide.
- It shape should be tongue shaped.

Precautions:

- Blood sample should be properly mixed by rotation before use
- The blood smear should be prepared from fresh blood /anticoagulant added blood preferably EDTA within 15 minutes of blood collection
- The smear should never be dried by blowing but rapidly dried by waving.
- The blood smear requires transportation delay in staining for more than a hour it should be fixed with methanol for about 15 min.
- Even the new slide be dipped in 95% alcohol and wiped dry with a clean cloth.

Staining of the blood smear:

Romanowsky type stain is composed of polychromed Methylene blue and eosin. methylene blue stain is a basic dye and eosin is an acidic dye.

EXAMINATION OF BLOOD SMEAR

A. Inspect the blood smear under low power magnification to note the distribution of cells, and select a portion of the smear near the thin end referred to as counting area, where the erythrocytes do not overlap but is present in considerable number. Switch to the oil immersion

objective for making the rest of observation. Also scan the feathered edge for platelet clumps, abnormal cells and parasites.

B. Inspect the erythrocytes in regard to size, shape, colour and other abnormal conditions.

C. Examine carefully the blood smear for platelets. These can be found scattered or in clusters in a blood smear. Presence of clumps or 7-10 platelets per oil immersion field is considered adequate. Always check for clumps in the feathered ends. These are cytoplasmic fragments of megakaryocytes. Mostly these are rounded structures with purplish granules. Some platelets have thread like processes denoting anaemia.

D. The differential count can be made by counting and classifying different 100 leucocytes in well stained blood smear.

E. smear should be carefully observed for the presence of intracellular or extracellular haemoprotozoans.

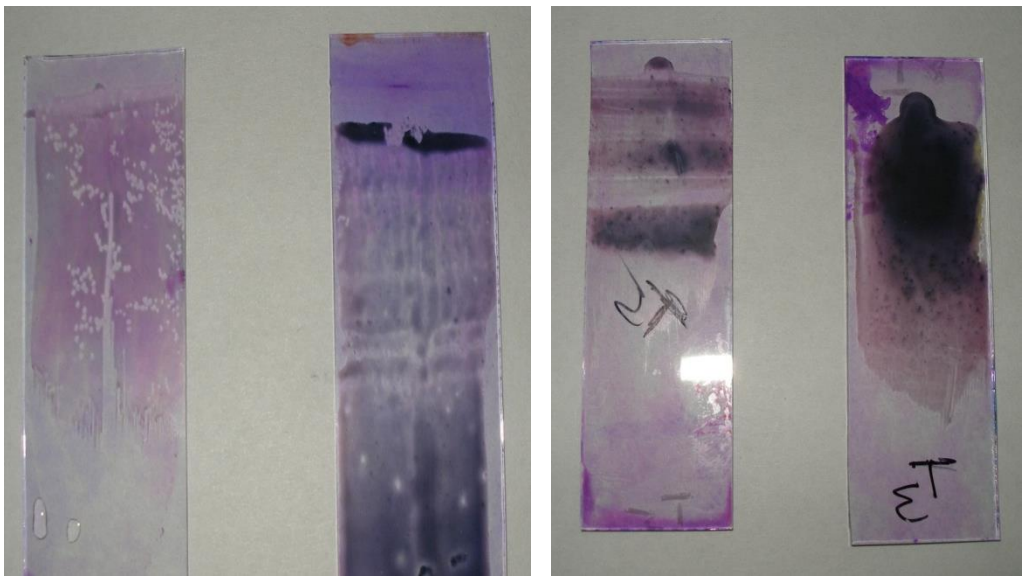


Fig. Improper blood smears showing greasy slides and un-uniform thickness.

STAINING

LEISHMAN'S STAIN: Prepared solution contains small amount of absolute methyl alcohol so, prior fixation is not needed. It is recommended for differential cell count but has the limitation that it does not stain the cytoplasmic granules well.

-flood the air dried blood film with undiluted stock Leishman's stain and leave for 1-2 min. to fix.

-dilute the stain on smear with double the volume of buffered distilled water and stain for 5-15 min.

-agitate the slide gently to aid mixing.

-wash with distilled water until the film has a pinkish tinge (0.5- 2min.).

-wipe the back of slide to remove excess stain and allow to dry in an upright position.

- Examine under microscope under oil immersion by putting one drop of liquid paraffin/ cedar wood oil on blood smear

GIEMSA STAINING: It is excellent stain for many blood parasites and for inclusion bodies, but neutrophilic granules and erythrocytes are poorly stained.

Prepartion of buffer:

a. KH_2PO_4 (Potassium dihydrogen orthophosphate)-3.0 gm

b. $Na_2HPO_4.12H_20$ (Disodium hydrogen orthrophosphate)-15.0 gm

Preparation of working solution**:** 1 ml giemsa stain + 9 ml neutral distilled water or buffer solution

Procedure:

- Make a thin blood smear.
- Fixation- fixes the blood smear in methyl alcohol for 1-2 minutes by pouring or flooding smear with methyl alcohol over. Fixation prevents autolytic degeneration i.e. blood cells may shrink or stretch due to osmosis or to be digested by their own cellular enzymes.
- After fixation, air dry the smear

- Slide is then kept on a staining rack and sufficient diluted Giemsa satin (1:9) is poured on the blood smear and left for 45 minutes
- After 45 minutes, wash the stained blood smear with neutral water till the smear is just pink
- Drain the excess stain, wash, dry and examine under microscope under oil immersion.

WRIGHT'S STAIN: It is good for routine haematological procedures.

- Flood the dried blood smear with Wright's stain and allow to stand for 1-3 minutes.
- Dilute the stain with equal volume of buffered water (pH 6.6-6.8) ensuring equal distribution over smear and solution should not run over the edge and allow to stand for 5-10 minutes.
- The metallic scum turned green is flooded with tap water and wipe off excess stain from both side of slide.
- Dry and examine.
- Count the cells under oil immersion objective (first examine under low power magnification for even distribution cells) along the side margins/top margin where erythrocytes do not overlap.
- Neutrophils are present along the margins; lymphocytes are more near short distance from margin and monocytes/eosinophils evenly distributed throughout. The morphology of various cells is given in table and is prerequisite for counting.
- The 100 cells are counted by Meander/Bettlement method involves examining 3 field along the margin, 3 field inward of margin, 3 filed parallel to margin and back 3 fields towards margin and procedure is repeated till 100 cells are counted .
- The cells are counted to 100 using specialized counter/hand tally method.
- The differential leukocyte count is expressed in per cent of the relative number of different leukocytes present in blood. Calculate the absolute count of various leukocytes using total leukocytes count and dividing by hundred.

Precautions:

- The diluted Giemsa stain must be prepared fresh as it is stable only for a few hours.
- The Wright's stain should be filtered before use.
- The same container can be used for diluting giemsa stain as residual stain is desirable
- Do not allow stain to dry in Wright's/Giemsa
- Washing should not be more.

Note- For Reticulocyte staining vital stains like New Methylene blue or Brilliant cresyl blue is needed.

Defects in staining of slides:

Defect	**Cause**
1. Entire blood smear is too blue, granules of eosinophils overstained and larger. .	- Too alkaline buffered water/wash water/stain - Excessive thick smear - Alkaline residue on slide - Insufficient washing - Prolonged staining before diluting with buffered water
2. Leukocytes nuclei pale blue R.B.C. and eosinophilic granules unusually red.	-Too acidic buffered water/wash water/ stain - Acid residue on slide.
3. Entire smear has pale stain	- Under staining. - Weak stain. - Excessive washing. - Using warm/hot water for washing slide.
4. Variation in staining on different areas of smear	- Buffered water unevenly applied or not thoroughly mixed - Acid/alkaline residue on slide - Water not properly drained from slide after washing
5. Precipitated stain	- Lack of thorough washing - Precipitate in Wright's stain (not properly filtered) - Evaporation of alcoholic stain due to excessive staining time. - Tilting of slide to one side and stain runs down

HAEMOGLOBIN ESTIMATION

Methods:

(i) Direct matching method- A. Tallquist haemoglobin scale

B. Dare haemoglobinometer

(ii) Oxyhaemoglobin method.

(iii) Cyanmethaemoglobin method.

(iv) Sahli's acid haematin method-best method.

Principle:

The basic principle involved includes haemoglobin conversion to acid hematin using dilute hydrochloric acid and matching the developed colour with comparator/standard colorimeter.

Requirements:

Sahli's haemoglobinometer. It consists of following-

1. Comparator of brownish/ amber coloured.
2. Sahli's haemoglobinometer glass tube with marking of gram percent/percentage of hemoglobin.
3. 0.1 N (N/10) HCl
   -Distilled water
   -Glass stirrer
   -Sahli's pipette having marking of 20 mm.

Procedure:

(i) Mix the anticoagulants added blood in collection bottle by rotation.

(ii) Take HCl in Sahli's tube up to mark 10cm percentage side/mark 2 on haemoglobin side.

(iii) Draw the anticoagulant added blood upto mark 20 cu. mm. in pipette.

(iv) Wipe the blood from tip and if the blood is overs the mark, reduce by tapping with finger.

(v) Transfer the blood directly into the graduated tube containing HCl. Suck the contents in pipette and expel in the HCl several times.

(vi) The contents should be mixed by circular movements/stirrer and allow to stand for 5-10 min.

(vii) Dilute the mixture with distilled water drop by drop, mixing with stirrer till colour matches with colour of standard.

(viii) Recording the reading in Sahli's tube and record the concentration of Hb as g/100 ml of blood.

Precautions:

-Mix the blood sample by rotation.

- Wipe out extra blood from external surface of pipette.

-Allow sufficient time to complete chemical reaction.

Sources of error:

-Error due to individual variation to record colour matching.

-Instrument is simple and least expensive.

-Some inactive form of haemoglobin such as methaemoglobin /sulfhaemoglobin / carboxyhaemoglobin in acid solutions are not converted into hematin and consequently not included in Hb concentration by this method.

PACKED CELL VOLUME (HAMATOCRIT) ESTIMATION

Depending upon the specific gravity of the blood components, high speed centrifugation separates blood into different components *viz.*

i. Dark red layer of red cell mass in the bottom.

ii. White to gray layer of leucocytes, thrombocytes (Buffy coat) just above the red cell mass.

iii. Fluid components i. e. blood plasma on the top.

iv. PCV is the most accurate, simple and inexpensive method for the detection of degree of anaemia.

Methods:

(i) Wintrobe method

(ii) Microhaematocrit method

A. WINTROBE METHOD:

Requirements:

-Anticoagulant added blood sample.

-Wintrobe tube-Flat bottomed tube having marking of 10 mm scale which read up 0- 10 on right side and used for PCV determination while left scale read down 0-10 cm and used for erythrocyte sedimentation rate.

-Dry syringe with 5-6 inches blunt needle (16-18 gauge).

-Centrifuge.

Procedure:

-Mix the blood sample thoroughly by circular movements for uniform suspension of cells.

-Take the blood in dry syringe.

-Insert the needle to the bottom of tube, release blood and simultaneously withdraw the pipette slowly avoiding formation of bubbles and keeping tip below the level of blood.

-Fill the tube to mark 10 on the right side.

-Centrifuge at 3000 r.p.m. for 30 min.

-Read the scale on right side at the top of packed erythrocytes immediately below buffy coat.

-Multiply reading by 10 and express as percent.

* approximate value of total leucocyte can be calculated by buffy coat using the calculation that every millimeter of buffy coat equals to 10,000 and thereafter every 0.1 mm equals 2000WBC's/µl. There is possibility of error in case of thrombocytosis.

Disadvantages:

-More blood sample is required.

-More time is required.

B. MICROHAEMATOCRIT METHOD

Requirements:

(i) Anticoagulant added blood sample.
(ii) Capillary tube (7 cm in length and 1mm bore).
(iii) Sealing clay.
(iv) Microhaematocrit Centrifuge.
(v) Haematocrit tube reader.

Procedure:

- Mix the anticoagulant added blood sample by rotation.

- Fill up the tube upto 2/3 rd level by Capillary action by holding tube in horizontal plane.
- Seal the free end of Capillary tube by Sealing clay and tube is placed in groove in centrifuge taking care that sealed end is away from central hub.
- Run the centrifuge (after closing the cover) for 5 minutes at 12000 rpm or 2 minutes at 16,000 rpm.
- Read the level of PCV by special haematocrit reader express it in percentage.

Advantages:

-Small quantity of blood.

-Shorter time.

-Greater accuracy.

Disadvantages:

-Special haematocrit reader is required.

-ESR and buffy count estimates cannot be made.

Note:

1. Estimated Hb. (%) = PCV/3 and Estimated RBC's (millions)= PCV/6.
2. PCV less than normal indicates anaemia while more than normal indicates haemoconcentration/ polycythemia.
3. Normal plasma is straw coloured while
   i. deep yellow is indicative of Jaundice
   ii. pale plasma is indicative of excessive blood loss and bone marrow depression
   iii. reddish is indicative of haemolysis
   iv. creamy top is indicative of of lipemia.

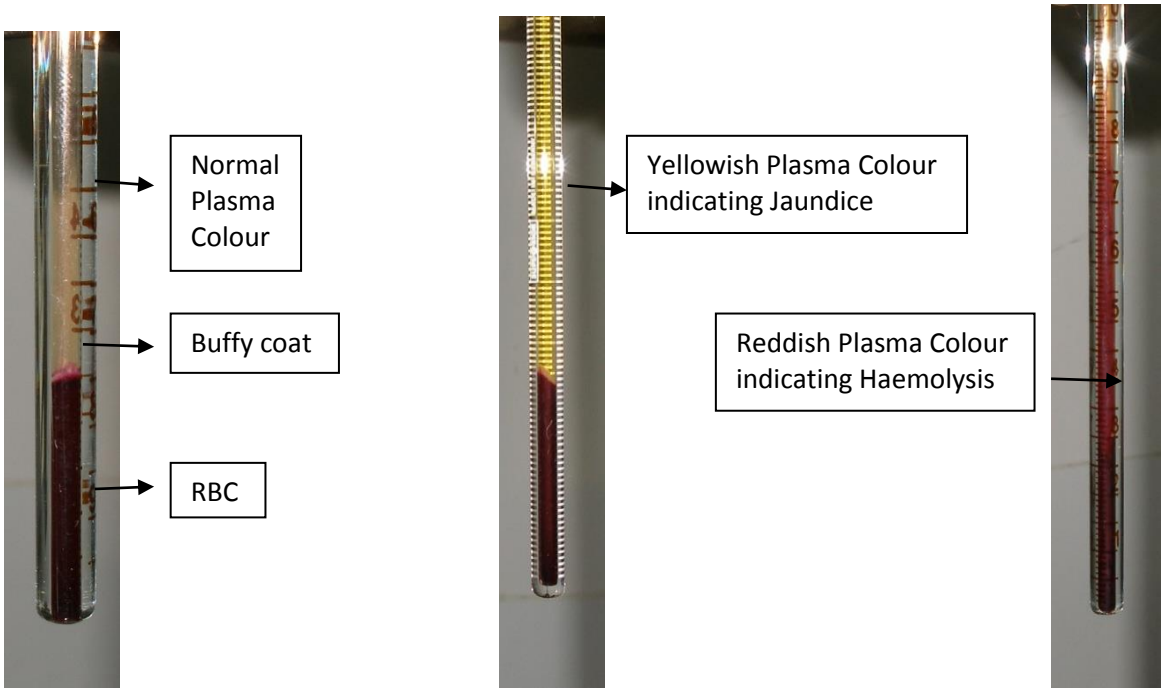


Fig. Haematocrit demonstrating centrifuzed blood.

ERYTHROCYTIC SEDIMENTATION RATE ESTIMATION

The distance by which the erythrocytes fall during a given period of time is measured in millimeter (mm) and is known as ESR.

Principle:

A tube containing blood to which an anticoagulant has been added is place in a vertical position; erythrocytes sink because they are heavier than the plasma in which they are suspended. The distance that the erythrocytes fall during a given period of time is measured.

Methods:

(A) Wintrobe tube method
(B) Westergren method

A. WINTROBE TUBE METHOD:

Requirement:

- Anticoagulant added blood sample.
- Dry syringe with 4-6 inch long needle (16-18 guage).

- Wintrobe tube with steel rack.

Procedure:

- The wintrobe tube is filled to the 0 mark on the left scale.
- The tube is placed vertically in appropriate rack.
- The upper level of sedimenting, erythrocytes is measured in mm on the left scale at time interval for particular animal species as detailed below:

Buffalo/Horse -10, 20 or 30 min
Cattle - 8 or 24 hours
Sheep & goat - 24 hrs
Dog, cat & man - 1 hour
The results are expressed as fall of R.B.C.'s in mm/hr.

B. WESTERGREN METHOD:

Requirement:

- Blood sample containing anticoagulant.
- Westergren tube with stand.

Procedure:

- Fill up the Westergren tube (total length of 300 mm, 2 mm diameter, a capacity of 1ml and graduation from 0-200 at 1mm intervals) upto the 0 mark by aspiration.
- The tube is fixed in upright position in a special rack with a soft rubber cushion at the bottom so that the tube is sealed when inserted.
- Record the fall of erythrocytes in mm at various intervals as mentioned in wintrobe tube method.

Sources of error and precautions:

- The sample should not be too old (more than two hours) and should not contain excess of anticoagulant.
- Refrigerated samples should be brought to same temperature since temperature changes effect ESR & vice-versa.
- The wintrobe tube should be perfectly perpendicular.
- Trapping of air bubbles should be avoided since it affects the rate.
- The tube should be placed in an area free from any disturbance/vibrations.
- The tube should be clean and dry.
- The blood sample should not be haemolysed.

INTERPRETATION OF ERYTHROCYTE SEDIMENTATION RATE

It is a non-specific test to determine the intensity of disease process in body.

1. Normal ESR does not exclude the possibility of disease.
2. It is more useful in dogs, cats and pigs and not useful in horses and buffalo (very rapid because there is an intense rouleax formation). Rouleax formation (clumping of RBC's) is seen only to mild degree in cat, pig and dog and not seen in cattle, sheep and goats.

COUNTING OF BLOOD CELLS

TOTAL ERYTHROCYTE COUNT ESTIMATION

Methods:

A. Haemocytometer method – Cheap and the best.
B. Electronic counting method – High cost.

HAEMOCYTOMETER METHOD:

Principle:

Accurate dilution of a measured quantity of blood with a fluid which is isotonic with the blood and count erythrocytes in counting chamber.

Requirements:

i. Blood samples with anticoagulants.
ii. Thoma diluting pipette containing red bead in the bulb. Graduated from 0.5-101.
iii. Diluting fluids:

A. Normal saline.
B. Hayem's solution:

Mercuric chloride	0.5mg
Sodium chloride	1.0g
Sodium sulphate	5.0g
Distilled water	200 ml

There may be agglutination in blood when the solution becomes more than 2-3 weeks old.

C. Grower's solution

Sodium sulphate anhydrous	12.5g
Glacial acetic acid	33.3ml
Distilled water	200 ml.

Counting not good as agglutination is common.

Procedure:

i. Mix the blood sample with anticoagulant by rotation.

ii. Draw blood in RBC diluting pipette up to 0.5 mark and excess blood be wiped from outside and if it cross the mark, the level is brought up to mark by tapping with the finger.

iii. Draw diluting fluid up to 101 mark, detach rubber tubing and mix the contents of pipette for 2-3 min. by holding horizontally between the thumb and middle finger.

iv. Discard at least 1/3 part of the contents of pipette and wipe off the tip (purpose is to remove fluid in capillary portion of the pipette which has not mixed with blood).

v. Load the haemocytometer by touching tip of pipette in space between counting chamber and the cover glass.

vi. Allow about 3 min. for the cells to settle but avoid evaporation.

vii. Count cells in central square of the 9 large square. Under high power, count erythrocyte in 5 of the 25 small squares in the central area following principle of double ruling or triple ruling.

viii. Variation of more than 10 percent in any of the central 5 squares indicates uneven distribution and require recharching of haemocytometer.

Calculation of erythrocytes/mm^3= Cells counted × 10 (0.1 mm depth) × 5 (1/5 of sq.mm.)× 200 (1:200 dilution) = Sum of cells in 5 small squares ×10,000.

ERYTHROCYTIC INDICES

It is related with size and Hb. Content of RBCs.

1. Mean Corpuscular Volume (MCV): It is calculated from the formula:

$$MCV\ (fl) = \frac{PCV \times 10}{TEC\ (million/\mu l)}$$

Increases in macrocytic anaemia and decreases in microcytic anaemia due to Fe or Cu deficiency.

2. Mean corpuscular haemoglobin concentration (MCHC): It is calculated by:

$$MCHC\ (g/dl) = \frac{Hb.\ (g/dl) \times 10}{PCV}$$

MCHC decreases in hypochromic anaemia. Never increases.

3. Mean corpuscular Heamoglobin (MCH):

$$MCH = \frac{Hb.\ (g/dl) \times 10}{TEC\ (million/\mu l)}$$

More valuable measurement for Anaemia.

RETICULOCYTE COUNT

These are immature RBCs. The reticulocyte concentration helps in evaluation of anaemia. The basis of reticulocyte count involves the events in the maturation of erythroid cells. Reticulocytes differ from RBC in having larger size, polychromatophilc and no rouleaux formation. Normally 1% reticulocyte is seen. It retains basophilc substance and are stained by supravital stain like neo-methylene blue or brilliant cresyl blue. Mix several drops of blood are added to stain in a test tube. It is incubated for 10 min. and then taken out to prepare a blood a blood film. RBC takes light stain whereas reticulocytes tyook blue colour. A total of 1000 red blood cells (RBC and Reticulocyte) are counted. From this the percentage of reticulocyte is derived. Toatl number of reticulocyte per cubic milliliter is calculated by multiplying the erythrocyte concentration by the percentage of reticulocytes.

Reticulocytes/µl = RBC/µl × % Reticulocytes.

INTERPRETATION

Is most useful in dogs and cats. It has some usefulness in cows. Increased concentration indicates regenerative anaemia while decreased number indicates aplastic anaemia.

TOTAL LEUKOCYTE COUNT DETERMINATION

Total number of leukocytes in peripheral blood in a given unit of blood is total leukocyte count.

Methods:

1. Electronic counting methods - Costly
2. Packed cell volume methods - Give only approximate counts.
3. Haemocytometer methods - Cheap and the best.

HAEMOCYTOMETER METHOD:

Requirements:

- Anticoagulant added sample.
- Haemocytometer with special cover glass.
- WBC diluting (0.5 & 11 mark and white bead in centre).
- Microscope.
- Diluting fluid.

Purpose is to haemolyse the erythrocytes.

Following diluting fluids can be used

i. 0.1 N (N/10) HCL.

ii. Turk's fluids:

Glacial acetic acid	- 2ml
Gentian violet (1% aqueous)	-1ml
Distilled water	-100ml

Filter before use.

Procedure:

- Mix the blood sample by rotation.
- Take the blood in WBC diluting pipette (after attaching with rubber tubing) upto 0.5 mark and wipe blood from the outside.
- Draw diluting fluid steadily to the 11 mark above the blood.
- Remove rubber tubing and mix the content the pipette keeping it horizontal for 2-3 minutes.
- Discard first 2-3 drops (1/3 part) from the pipette before filing the counting chamber.
- Load the haemocytometer by touching the tip of pipette in space between edge and cover glass.
- Allow leukocytes to settle for 2 minutes and also for lysis of erythrocytes.
- Count the cells under low power magnification (x10) in four corner large squares (each is divided in 16 small squares) by following the exclusion principle of cells counting along the line as discussed in TEC.

Calculation:

$$\text{TLC/mm3} = \frac{\text{Cell counted} \times 20 \text{ (1:20dilution)} \times 10 \text{ (0.1 depth)}}{\text{4 (No. of sq. mm. counted)}}$$

$$= \text{Sum of white blood cells in squares} \times 50.$$

Source of error and precautions:

- Almost same that TEC.
- If TLC is more (50,000 to 5, 00,000) use R.B.C. diluting pipette for more dilution.

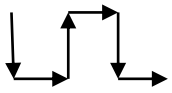
- If variation in counting of cells is more than 10 per cent in full squares counted the procedure should be repeated and the haemocytometer may be recharged.

DIFFERENTIAL LEUCOCYTE COUNT

The differential leucocyte count is done by counting different 100 leucocytes in oil immersion field. However, distribution of leucocytes in the smear is checked initially on high power. The counting is done on the feathered edge of the blood smear. The site where RBC's remain scattered but neither scarcely populated and also not in large clumps is preferred for counting. The counting is done by Battlement method. Here, counting is started from the periphery of the selected site for about three fields then move inwards at right angle towards the middle portion for about three fields, again move straight to the edge and then move upwards making parallel lines. Repeat it as many times as necessary to count at least 100 cells. The report is provided as the percentage of different leucocytes. Identification of different types of WBC's is done on the basis of Size, Cytoplasm (colour, granules) and Nucleus (shape, lobes, chromatin structure). Also record for any variation in morphology of cells. Usually 2 to 3 cells per field are considered normal count.

Source of error and precautions:

- Care should be taken to choose the right site for cell count.
- Too thick area selected will not give proper picture as overlapping may occur.
- Too thin area will have little cells and counting of 100 cells will be cumbersome.
- Never restrict your counting to the edge only as neutrophils tend to abundant at the margins.
- Never restrict your counting to the sub periphery only as lymphocytes tend to abundant below the margins.
- Monocytes and eosinophils are evenly distributed.
- The central area of slide is usually devoid of cells.



Battlement method of counting

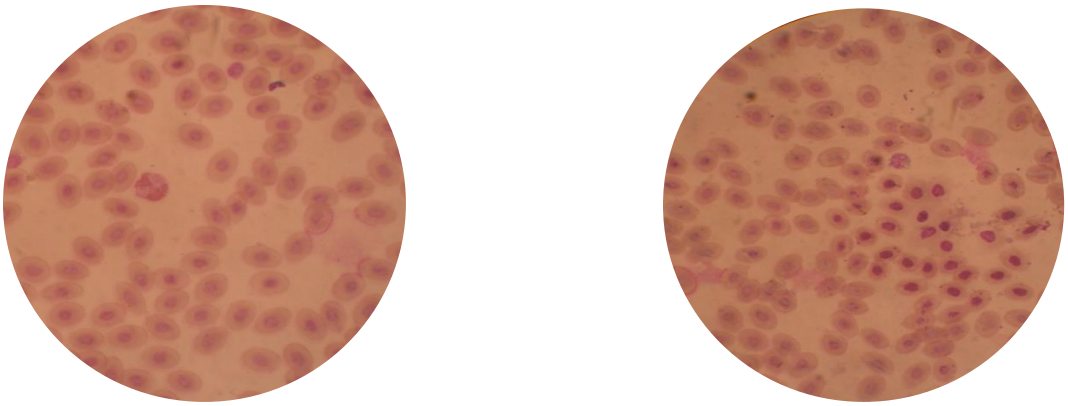
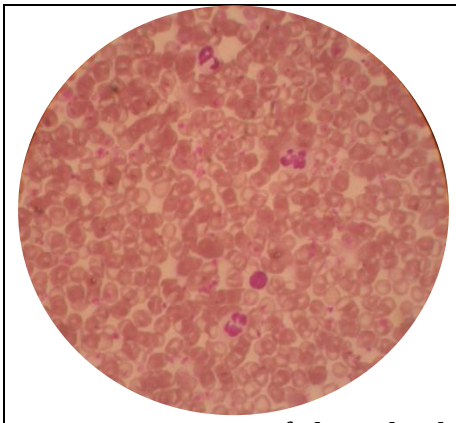
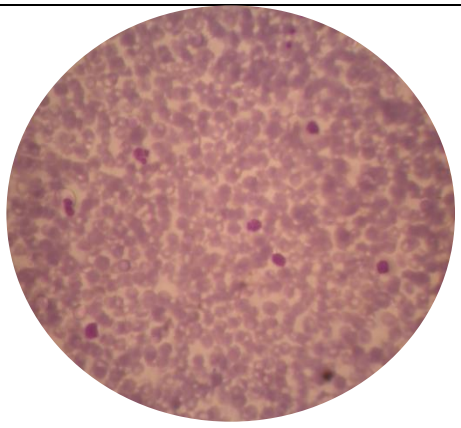


Fig. Microscopic view of Poultry Blood showing nucleated RBC and neutrophils.



Microscopic view of dog Blood showing RBC's, Lymphocyte and neutrophils.

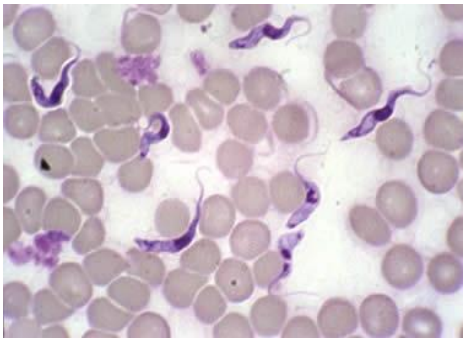


Microscopic view of Cattle Blood showing RBC's, Lymphocytes and neutrophils.

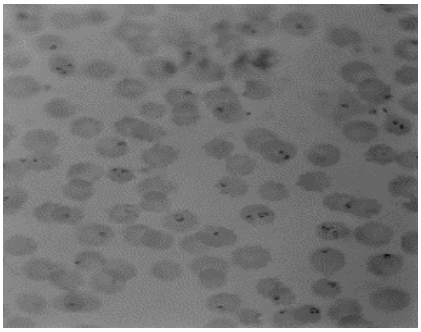
THROMBOCYTE COUNT

Thrombocytes are generally seen in clusters but they appear as single or in clumps. The cytoplasm appears light with deep reddish purple staining granules. 4 or more thrombocytes per oil immersion field are considered normal. Normal range varies from 200.000 to 500,000/microlitre. Thrombocytopenia can be seen in Aplastic anaemia, leukemia, systemic fungal infection, equine infectious anaemia, Ehrlichiosis etc. It is manifested by puprpura and petechiation.

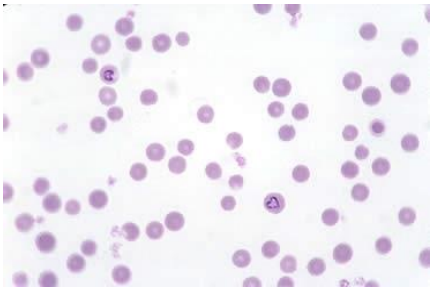
PHOTOGRAPHS



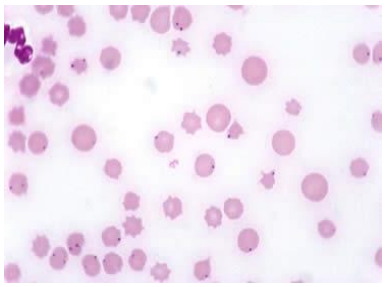
Trypanosoma



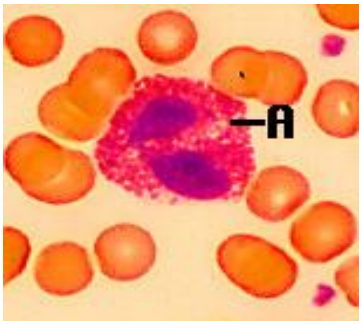
Theileria



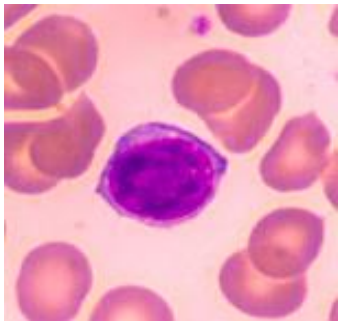
Babesia



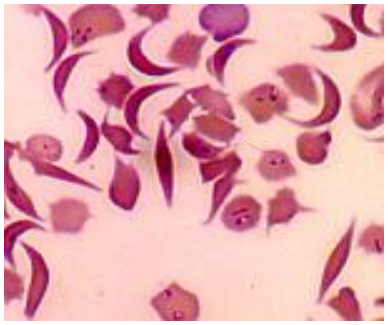
Anaplasma



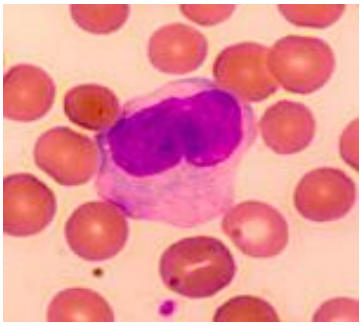
Eosinophils



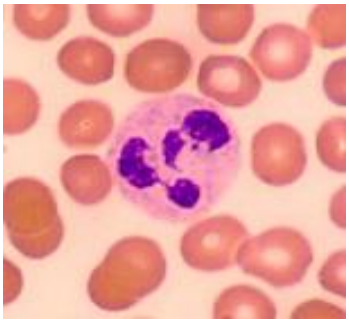
Lymphocytes



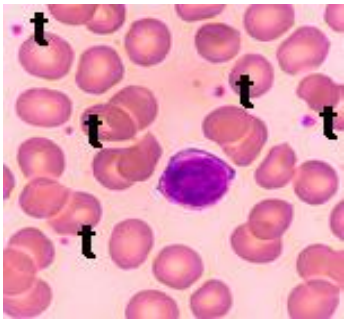
Sickle cell R.B.C.



Monocyte



Neutrophil



t=Platelets