

Practical hematology

Blood sampling	
Small amount	Large amount
Puncture of the comb	Wing vein Inside the elbow joint
Cutting the tip of comb	Tibial vein Just above the knee joint at medial aspect
Cutting of toe nail	Shank vein (mainly in duck and geese)
	Jugular vein (right side is larger vein)

Anticoagulants

EDTA:

Dose: 1-2 mg/ml blood

But after 15 min blood become dark & viscous

Although the blood does not clot but the cells are disrupted enough to make sample useless

Heparin:

Is the best for test requiring plasma

Dose: 25 lu / ml blood

Avian Haematology

Avian blood is a sticky bright red coloured fluid, it represents about 6.5-8.5% of body weight.

Avian blood is composed of:

1-Plasma: Straw yellow in colour and slightly turbid.

2-Cellular elements:

A-Rbcs

B-Wbcs

C-Thrombocytes

N.B All cellular elements are nucleated

1-Plasma

1-It is a straw yellow in colour and slightly turbid in appearance.

2-Water constitute about 80% of plasma.

3-It contains various of compounds as proteins, hormones, vitamins ,glucose, Minerals, fatty acids

4-These compounds differ according to age, sex, egg production, type of foods, condition of animals

5-Plasma of layers contain 2 folds increase in calcium than non layers (9.3-12.3 mg%).

6-Plasma of layers contains 2-5 folds increase in plasma lipids than nonlayers (due to increased secretions of estrogen from ovary in layers).

7- Glucose concentrations in birds range from 126-204mg%

A- Glucose concentration drops with age.

B- Glucose concentration increase with hatching.

C- In Stress glucose concentration increase.

So generally, plasma glucose concentration in birds is higher if compared with mammals.

8-Plasma proteins ranges from 2.3-6.1 gm%

A-Plasma proteins of male is lower than female (for egg production).

B-The globulins are more concentrated in birds than in mammals,so birds are good antibodies producers.

9-Urates (uric acid):

A-Catabolic end products of protein and non protein nitrogenous compounds.

B-It ranges about 40 mg%

C-It is a vehicle by which amino-nitrogen is excreted.

Creatine & Creatinine

1-Creatine & creatinine are indicator for muscle metabolism.

2-Increase creatine & creatinine with increase uric acid indicates Muscle catabolism.

3- Increase creatine & creatinine with decrease uric acid indicate Muscle Anabolism.

Total RBCs and WBCs count

- Total RBCs and WBCs count can be directly counted from the same blood sample placed in the hemocytometer.

Apparatus

- Hemocytometer
- RBCs diluting pipette
- Diluting solution (Natt and Herrick)

Natt and Herrick

Composition

NaCl	3.88 g
NaSO₄	2.50 g
Na₂HPO₄.12H₂O	2.91 g
KH₂PO₄	0.25 g
Formaline (37%)	7.50
Methyl violet	0.10g

**Dissolved in Distilled water to a total volume =1000 c.c.
The solution has a pH =7.3**

Advantages of Natt and Herrick solution

Have osmotic pressure as that of chicken blood(do not distort blood cells)

Both total RBCs and WBCs count can be made from the same blood sample

Procedure

- **Blood till 1.0 mark in diluting pipette (dilution factor 1:100)**
- **Diluting solution till 101 mark (dilution factor 1:100)**
- **Allow the mixture to stand in the pipette 1-2 min**
- **Shake well for 1 min to obtain equal cell distribution**
- **Counting of RBCs and WBCs in the central primary square.**

Calculation

- **$\text{RBCs/mm}^3 = N/80 \times 400 \times 100 \times 10$**
- **$N =$ no of RBCs in five 2^{ry} squares**
- **$\text{WBCs/mm}^3 = N \times 100 \times 10$**
- **$N =$ no WBCs in whole 1^{ry} square**

Cellular elements

All cellular elements of birds are nucleated

A-Erythrocytes:

1-It is 10-15 μm , larger than mammals(5-7 μm), smaller than reptiles(18-23 μm).

2-Elliptical in shape, biconvex, nucleated, contain mitochondria, its life span is about one month.

3-Counted by haemocytometer

4-Their number varies from 1.5-6.6millions\mm₃.

5-Rbcs number depends upon :

Age: is higher in young animals

Sex: Male is higher than females

Hormones:

Anabolic hormones as thyroid hormones increase number of Rbcs.

Estrogen interfere iron absorption so decrease Rbcs count.

Seasons:

Winter stimulate thyroid activity so increase Rbcs count .

Altitude:

High altitude increase Rbcs due to increase Erythropoitin hormones.

Erythrocytes osmotic pressure :

O.P of birds is higher than in mammals (1.02 Nacl)

B-Leukocytes

As in mammals there are Granular and Agranular leukocytes.

Number of leukocytes:

Chicken: 19-30 thousands/mm³

Duck: 32 thousands/mm³

Differential Leukocytic count

Preparation of blood smear

1-By using The Standard two-slides wedge technique as in mammalian blood.

2-Uncoagulated blood or EDTA-blood should be used and avoid heparin since it interferes with the proper staining of the blood cells.

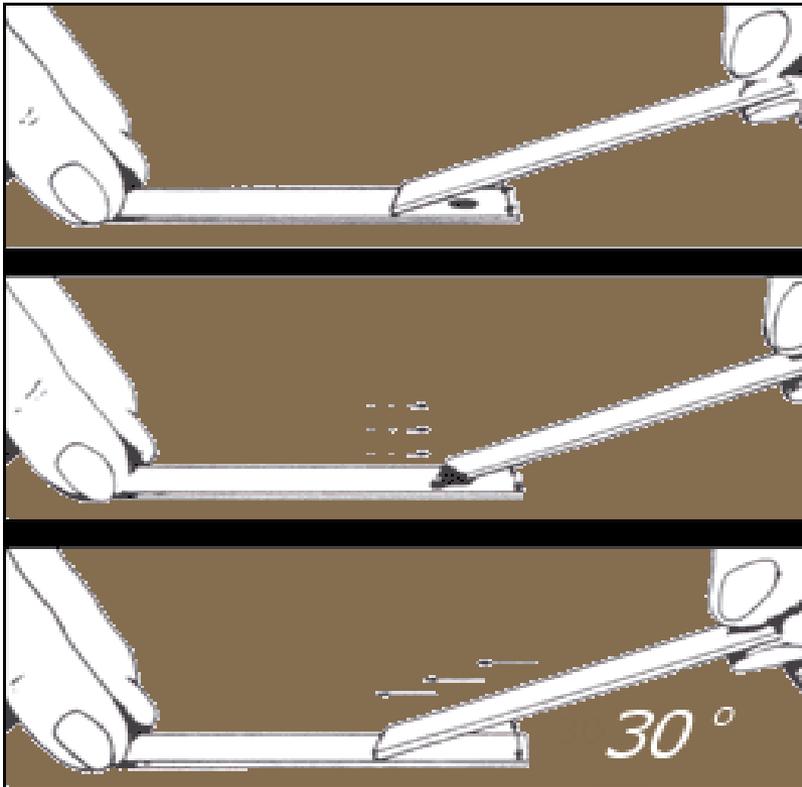
The commonly used stains Are:

A-Wright stain.

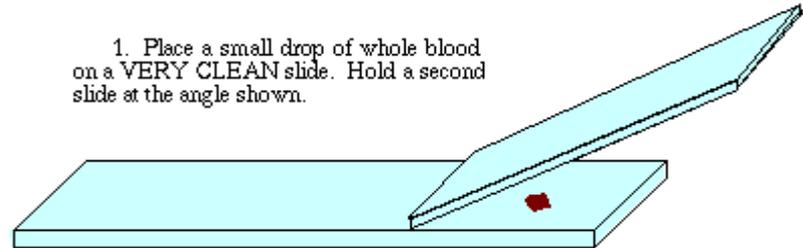
B-Wright –Leishman stain.

C-Wright – Giemsa stain.

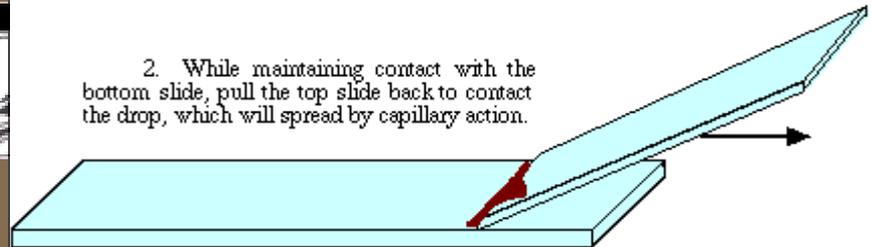
Blood smear



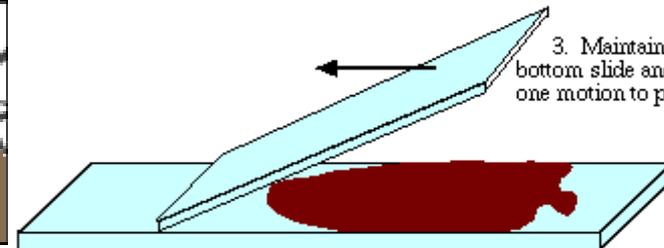
1. Place a small drop of whole blood on a **VERY CLEAN** slide. Hold a second slide at the angle shown.



2. While maintaining contact with the bottom slide, pull the top slide back to contact the drop, which will spread by capillary action.



3. Maintain firm contact with the bottom slide and push the top slide in one motion to produce the smear.



Leukocytes

Leukocytes are either granular or Agranular.

Granular leukocytes

- 1-Heterophils
- 2-Eosinophiles
- 3-Basophils

Agranular leukocytes

- 1-Lymphocytes
- 2-Monocytes

1-Heterophils

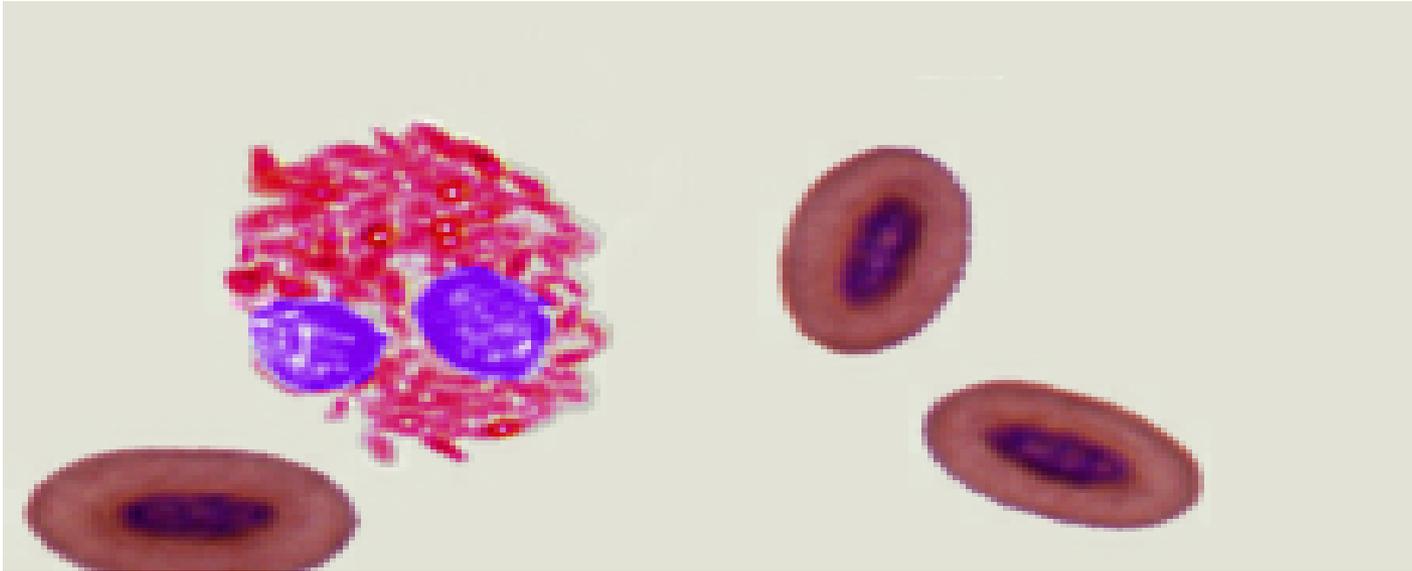
1-It is analogue of mammalian neutrophils .

2-Its diameter about 10-15 μ .

3-The nucleus is polymorphic with varying degree of lobulation.

4-The cytoplasm contains a characteristics rod-like granules , pointed at their ends, acidophilic bodies.

The percentage of heterophil in blood ranges 20-30% (except in ostrich and pheasants 60-70%)



Heterophils are phagocytic and use their enzyme-containing granules to lyse ingested materials.

Heterophils are motile and can leave blood vessels to engulf foreign materials

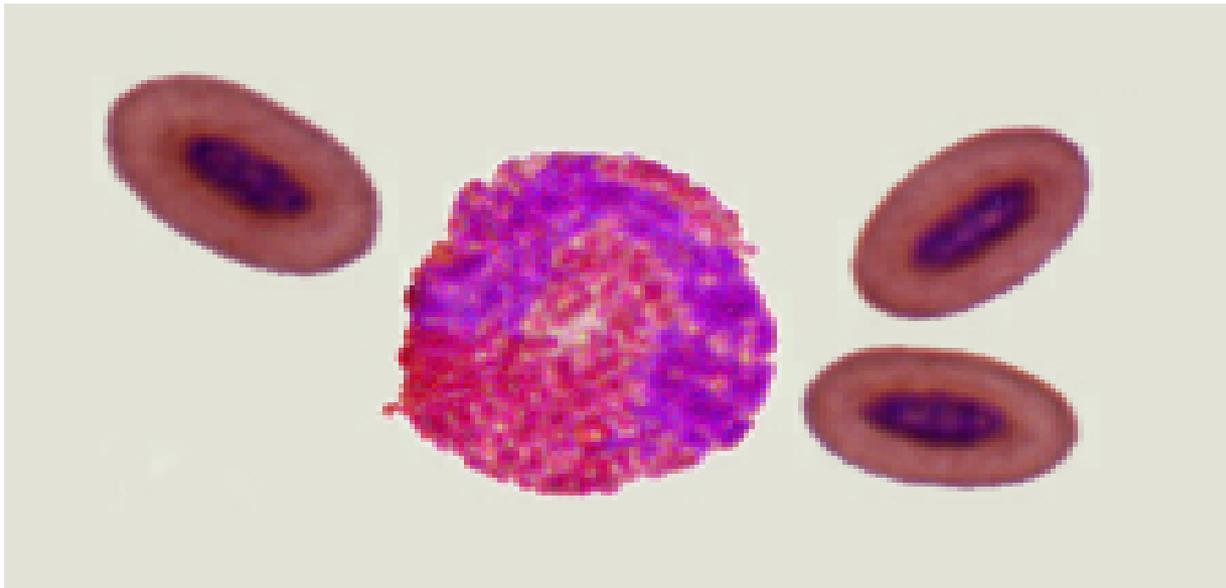
2-Eosinophils

1-The size of eosinophil is about the same size as the heterophil.

2-Rounded cells , The cytoplasm contains large, spherical, dull red coloured granules.

3-The cytoplasm is Faint blue colour.

4-The nucleus is often bilobed and Darker than that of heterophils.



Eosinophils make up about 2 to 3 % of the WBC population of healthy birds.

3-Basophils

1-The size of basophil is about the same size as the heterophil.

2-Rounded cells , the cytoplasm is devoid of colour and contains basophilic granules which may mask the nucleus and protruded from the surface of the cells giving black berry appearance.

3-The nucleus is weakly Basophilic , may be rounded or oval.

Basophils



4-Lymphocytes

1-The lymphocytes constitute the majority of the leukocytes in the blood of the fowl.

2-The cytoplasm is weakly basophilic , it may forms a narrow rim around the rounded nucleus in small lymphocyte.

3- The cytoplasm may constitute the major portion of the cell, as in the large lymphocytes. The nucleus is usually round and has a fairly coarse pattern of chromatin.



The percentage of lymphocytes in blood ranges 60-70% (except in ostrich and pheasants 20-30%)

Lymphocytes are either T-lymphocytes (formed in the thymus) or B-lymphocytes (formed in the bursa of Fabricius).

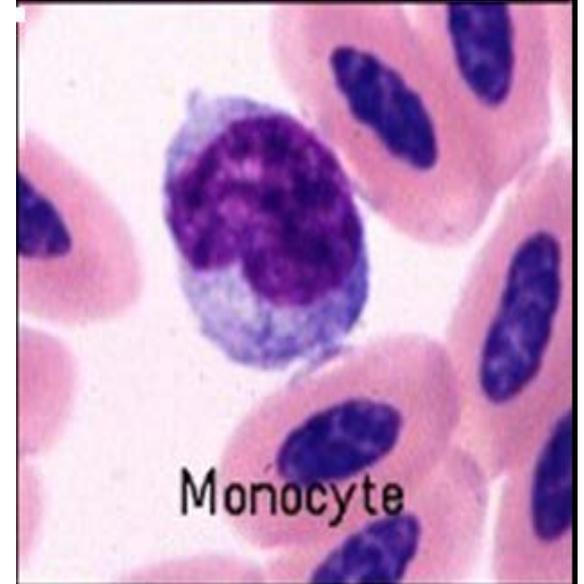
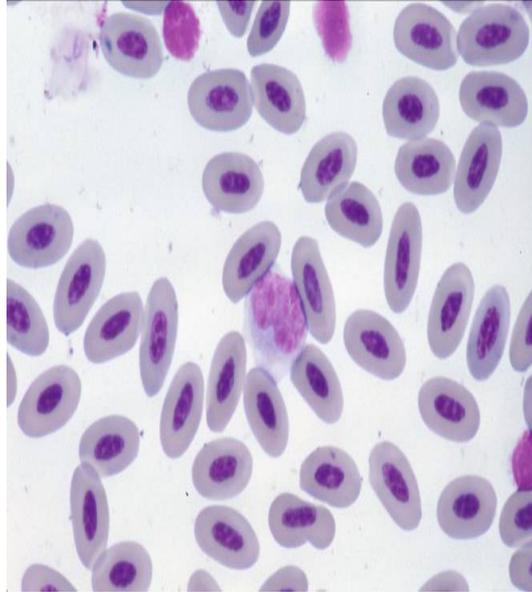
B-lymphocytes produce antibodies; T-lymphocytes attack infected or abnormal cells.

5-Monocyte

1-Monocytes are large cells with relatively more cytoplasm than the large lymphocytes.

2-The cytoplasm of these cells has a blue-gray colour and vacuolated.

3-The nucleus is usually irregular in outline



Monocytes %=5-10%

Monocytes are motile cells that can migrate using ameboid movements.

Monocytes are also phagocytic.

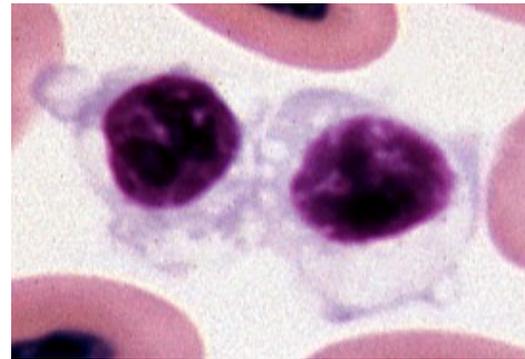
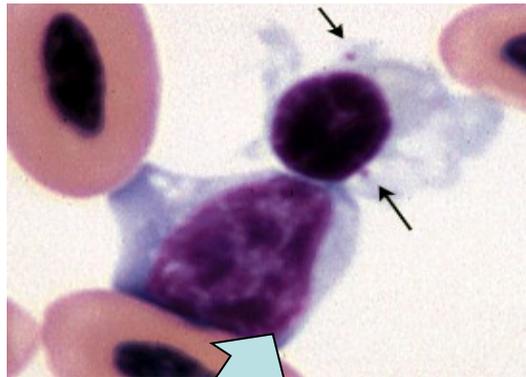
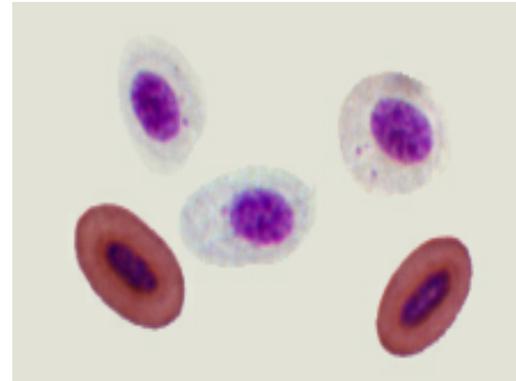
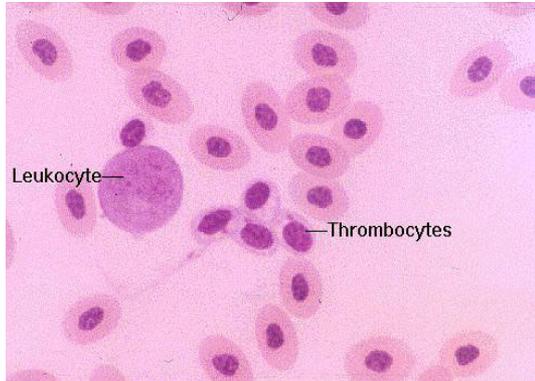
Thrombocytes

1-They are rounded to oval in shape and usually present in clumps.

2-They have rounded very darkly stained nucleus and a clear cytoplasm.

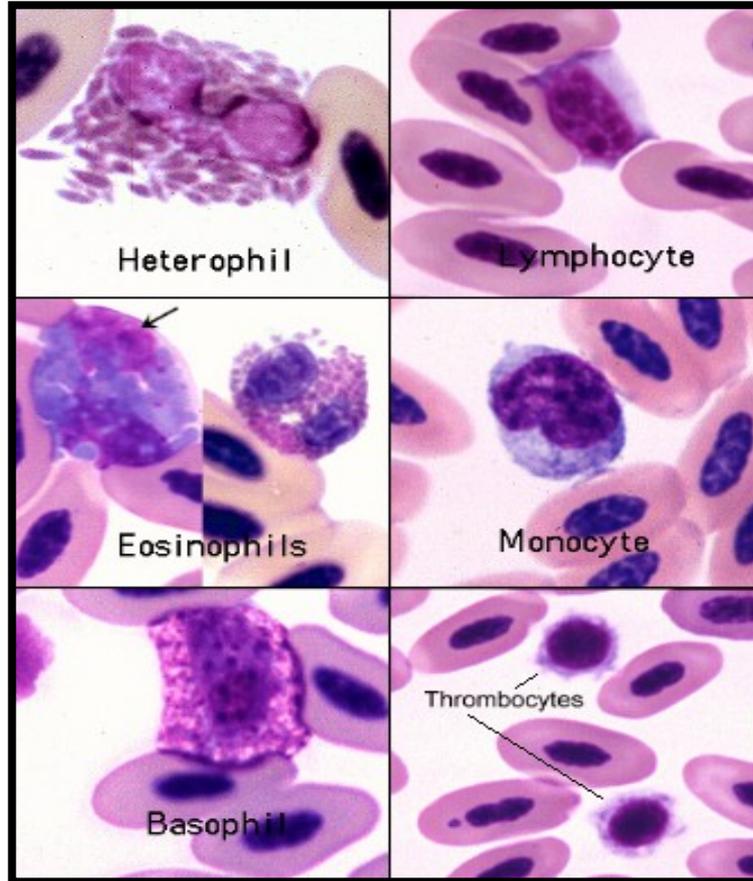
3-Thrombocyte are active participants in blood coagulation.

In addition to blood clotting, these cells may have the ability to phagocytize foreign materials (e.g. bacteria) and they are able to carry O₂ like RBCs if an extreme anemic condition exists.



**The avian thrombocyte (upper cell) usually has a smaller, more densely-stained nucleus compared to the lymphocyte (lower cell).
Thrombocyte cytoplasm tends to be paler in color, and more "ragged" or vacuolated in character compared to that of the lymphocyte.
Some thrombocytes contain small reddish granules (arrows).**

Leukocytes



Total thrombocytic count (TTC):

an estimate of thrombocytes number can be obtained using blood smears prepared as for differential count

Calculation:

If the PCV is within the range of 40-50%:

Estimated TTC/ ul = number of thrombocytes in 5 fields x 3500

If the PCV is outside the 40-50% range:

Corrected TTC/ ul = estimated TTC x (observer PCV/ normal PCV)

Comment:

Normally, there should be 2-3 thrombocytes (10-15/ 1000 RBCs) per microscopic field when viewed with the 100x objective under oil immersion.

Increased number of thrombocytes can indicate a chronic disease condition.

Haemoglobin Estimation

A-Modified Acid Haematin Method (Modified Sahli 's method)

Characterized by

1-The volume of HCL N/10 was used up to the 8th mark (% mark) instead of 10th mark (% mark).

2-The reading of Haemoglobinometer is multiplied by a factor 0.134 to obtain amount of Hb in gm%

For e.g.

85% (reading) x 0.134 = 11.4 gm%

B-Cyanomethaemoglobin method

Characterized by

1-The ordinary Drabkin 's reagent is also used.

2-The Turbidity formed after addition of blood on Drabkin 's reagents is removed either by:

A-Centrifugation of the tube containing the Drabkin 's reagent and blood to ppt. turbidity.

B-Passing a beam of natural gas or Co-gas through the tube.

3-Measurement calorimetric at W.L 540nm.

4-Blank solution : drabkin 's reagent.

Normal values of Hb

1-Chicken: 9.3 gm%

2-Ducks: 10.3 gm%

3-Turkey: 10.3 gm%

Avian Haemoglobin

1- In most vertebrates, hemoglobin is made up of four subunits, each one of which has its own binding site for oxygen.

2- There are some significant differences between avian hemoglobin and hemoglobin found in other vertebrates:

A- In adult birds there are two different types of hemoglobin, hemoglobin A and D, both of which vary from each other in their affinity for oxygen.

B- Hemoglobin A is often the more prevalent form and has a lower affinity for oxygen compared to hemoglobin D.

C- A lower affinity means that oxygen is more readily dissociated from hemoglobin as arterial blood

D- In general, avian hemoglobin shows more cooperativity with oxygen than does hemoglobin in other vertebrates.

E- Cooperativity is the phenomenon whereby the binding of one molecule of oxygen with hemoglobin facilitates the binding of the next molecule of oxygen and so on up to the binding of four oxygen molecules by a molecule of hemoglobin.

F- This cooperativity accounts for the sigmoidal shape of the oxygen-hemoglobin binding curve.

G- The advantage of this high cooperativity is that it increases the delivery of oxygen to tissues.

H- Another feature of avian hemoglobin is its interaction with inositol pentaphosphate and inositol tetraphosphate.

I- In mammals the principal organic phosphate is 2,3-biphosphoglycerate (2,3-BPG), formerly known as 2,3-diphosphoglycerate (2,3-DPG), which shifts the oxygen-hemoglobin dissociation curve to the right and decreases the affinity of oxygen for hemoglobin, enhancing the delivery of oxygen to tissues.

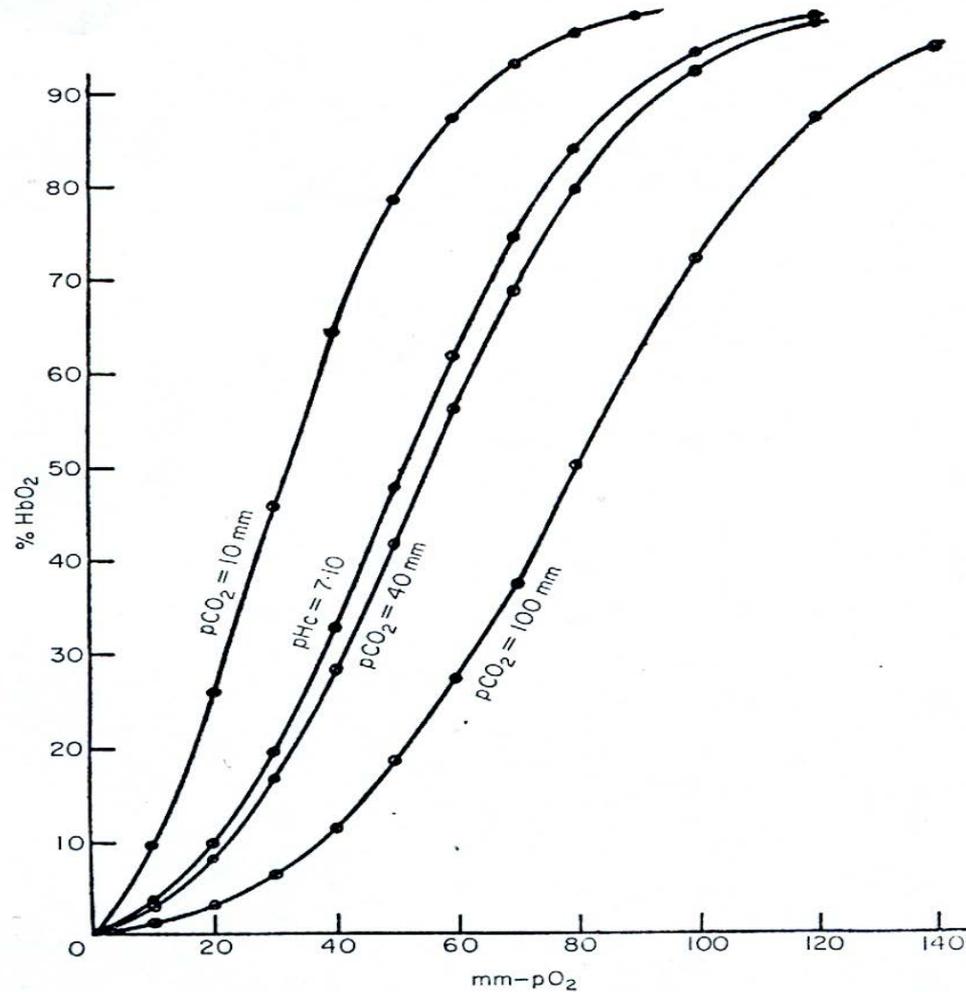
J- The presence of two hemoglobins with different oxygen affinities, means that the erythrocytes have a greater range of oxygen partial pressures over which oxygen can be bound and released, favoring the uptake of oxygen where there is little oxygen available in the environment (Fig. 8).

K- Carbon dioxide also affects the binding of oxygen to hemoglobin.

L- In mammals, the binding of carbon dioxide with hemoglobin is more so with deoxyhemoglobin than oxyhemoglobin, to form carbamino compounds, which result in small increases in P_{50} .

N- It appears that in birds the strong binding of the organic phosphates to hemoglobin prevents this carbon dioxide effect.

Fig. (8): Oxygen dissociation curves of adult fowls' blood at 40oC. Effects of variation of pCO2.



Erythrocyte sedimentation rate

Applied by a Westergreen 's method

Characterized by

A-The tube should be tilted at 45° angle ,as the vertical position is unsatisfactory for avian blood because avian blood cells settle very slowly.

B-Normal values:

ESR= 1-4 mm\hr

Packed cell volume (PCV)

A-Macrohaematocrite (Van-Allen Method)

1-Using Van-Allen Haematocrite tube

2-Centrifugation at 2000 r.p.m for 20 min.

(the oxalated-blood sample is filled into Van-Allen haematocrite tube till the mark 100% and then centrifuged at 2000 r.p.m\20 min.

3-The opened end of the tube is firmly closed by spring clip cap.

B-Microhaematocrite

By using Heparinized capillary tube

Tube is filled with blood then close with clay and centrifuge.

Normal values of PCV

Chicken	32%
Ducks	33%
Rabbits	41%

Coagulation screening tests for avian blood

1-Whole blood clotting time (CT)

Blood is collected into non-siliconized tube (2ml). Tilted every 15 seconds until a firm clot is observed

N.B.

Contamination of blood sample with tissue thromboplastin may shorten clotting time

2-Prothrombin time (PT)

Avian origin thromboplastin source is required for PT assay. Acetone-dehydrated brain powder have more thromboplastic activity.

Principle and procedure

Citrated plasma (tested sample) +
thromboplastin (of avian origin)
↓
(37-38.5oC)

Fibrin clot

(PT: time required for tissue thromboplastin to cause clotting of tested plasma/sec)

Normal for chicken: 9-11 sec

Recalcification time (RT)

Intrinsic pathway is evaluated with recalcification time

Principle and procedure

Citrated plasma (total plasma) + crushed glass (contact activator; XII XIIa)

(37-38.5oC)

(1-3 min)



+CaCl₂

(37-38.5oC)



Fibrin clot

(RT: time required for fibrin clot formation after additon of CaCl₂)

Activated partial thromboplastin time (APTT)

1-Intrinsic pathway is also evaluated with APTT, where platelet factor substitute prepared from chicken brain can be used as cephalin source.

2-Plasma must be preincubated with kaolin for 3 minutes before CaCl_2 is added.

Principle and procedure

Citrated plasma + cephalin of avian origin
+kaolin

(37-38.5oC)

(1-3 min)

(37-38.5oC)

+ CaCl_2

Fibrin clot

Comment

1-PT is a screening test used to monitor the extrinsic pathway of blood clotting.

2-RT and APTT are used for monitoring the intrinsic pathway of blood clotting.

3-Coagulation tests are prolonged in liver diseases, ingestion of vitamin K antagonists (e.g coumarin or warfarin), ingestion of ration contaminated with mycotoxins (aflatoxicosis).

4-Individual clotting factor assay.

5-Individual clotting factor assay

1-Individual clotting factor activity (e.g factor II, VII, IX, X and XI) can be measured.

2- The use of tissue thrompoblastin of avian source gives an accurate results.