INTRODUCTION:

Haematology

Clinical chemistry

Clinical microbiology & Parasitology

Urinalysis

Cytology/histology (biopsy)

GENERAL

In understanding the nature of a disease for effective treatment and control measures to be adopted, pathology must go beyond postmortem diagnosis to making use of changes in the structure and functions of organs/tissues in a living animal. Clinical pathology provides evidence regarding the physiological alterations resulting from pathological condition. Clinical tests make more meaning when considered in relation to the history of the patient.

Clinical chemistry-Application of chemistry and it's allied techniques for the elucidation of disease, diagnosis and management of patient.

Blood chemistry is of great value in some disease in conformation of diagnosis, prognosis and response to treatment {management of disease}. Interpretation of such analysis is done by comparing results with normal range values of blood constituents. Accuracy of such result depends on:

1) taking of appropriate samples in a proper manner.
2) availability of adequate information [history].

Clinical chemical analyses are mostly performed to determine parameters such as blood Glucose, body electrolytes and other metabolites, enzymes, proteins [including Ag & Ab] and renal functions.
Most clinical chemistry requires serum and occasionally plasma.

Blood for biochemical analysis will preferably require **heparin** as anticoagulant. This is because heparin is a natural anticoagulant produced by the liver and prevents the conversion of prothrombin to thrombin. **Ammonium oxalate** should

not be used as anticoagulant if blood non-protein nitrogen or urea is to be determined. The **oxalate** and **nitrates** [eg Na-citrate] combine with Ca^{++} to prevent clotting. While serum/plasma could be frozen for a limited period of time, depending on the required analysis, whole blood should not be kept frozen to prevent lyses.

For general haematological examinations the anticoagulant of choice is **EDTA** because it best preserves the cellular components and integrity as well as prevents platelets aggregation. Haematological examination are mostly preformed in the following areas:

1) Microscopic examination of unstained preparation
2) Microscopic examination of stained smears
3) Haemoglobin estimation
4) Packed cell volume determination
5) Red blood cell count
6) Total and differential leucocyte counts
7) Platelet count.
8) Clotting time and any other

BLOOD SAMPLE COLLECTION

Sample are generally taken for either haematological or biochemistry or serology while haematological examination are performed with unclotted whole blood.

Serological examinations are made with serum from clotted blood, while collecting blood, animals should not be excited unnecessarily. The site for blood sampling depends on the purpose and volume of blood needed before taking blood, the site should be sanitized by shaving [if necessary] and wiping the exposed skin with alcohol or either and allowing it to dry.eg sites and animal species good for blood sampling.

*Marginal ear vein-rodents and ruminants, pigs

*Jugular vein –for large quantities of blood –ruminants, horses, dogs

*subcutaneous abdominal vein –lactating cattle (anterior mammary vein)

*Middle coccygeal vein-cattle, pig

*Cephalic (radial) vein-cat, dog

*Recurrent tarsal vein-cat, dog

*anterior vena cava-pig

*Cardiac puncture-Rodent

Samples should be collected in perfectly dry container to avoid haemolysis. The superficial vein generally need to be occluded to distend them by application of pressure with the fingers or a suitable tourniquet for a brief time usually not long[2mins]. This should not be too tight. Sampling requires sharp and correct size of needle. Needles are inserted into the superficial vein at about 30 degrees to the skin.

Blood is withdrawn by gently applying negative pressure [traction] on the plunger of the syringe. Blood is delivered into the container after removing the needle. Selected anticoagulant is necessary in the container if unclotted blood is required. Adequate mixing is ensured by gently inverting the container several times immediately after sample is discharged into the container to ensure proper and uniform mixing. The choice of anticoagulant depends on the type of examination required. Blood smears should be made as soon as possible, since anticoagulants have effect on the morphology of the cells (rbc & wbc) when exposed to them over a long time.

The rbc (biconcave cells) are produced in the bone marrow of adult animals with the regulatory influence of erythropoietin, Cu, vit B_{12} and proteins etc. Erythrocytes are generally/normally non-nucleated cells in the mammals, nucleated in fish, reptiles & birds. The main function of the rbc is to convey O_2 to the cells/tissues by making use of haemoglobin molecules it carries.

Haemoglobin is made up of 2 main units: Haeme and globin. The haeme which isc a unit with iron carries oxygen to tissues. Pathology of the rbc therefore is mainly in the area of production, morphology & number of the cells and the nature of haemoglobin, ie. Haemoglobinopathy.

Erythropoiesis is a continuous process. The lifespan of a normal RBC is about

140-150 Days in horse

80 Days in Cattle

52Days in Sheep

62-70 Days in Pigs

110-120 Days in Dogs

68-77 Days in Cats

Normally millions of RBC are being removed from circulation every minute due to old age. An old RBC [senescent RBC]are removed from circulation either by in tissue eg spleen or a small percentage get lysed in circulation. In both cases, the iron past of the haeme is removed for reuse by the bone marrow. These normal produres could lso be disturbed in pathological cases.

Before being reused the iron moiety is locked in the macrophages as ferritin or haemosiderin when need they are released into circulation back to the BM.The remaining past of the haeme is converted in the liver to bilirubin.

Apast from the mature RBC which constitute about 95%/99% of the total erythrocytes there are few mature cells in circulation. Thes are reticulocytes (non-nucleated) but still with some RNA. Reticulocytes are better identified by special stains (supravital stains):

a) Brilliant cresyl blue in alcohol or saline for reticulocytes
b) New methylene blue
   giving an orange coloured cell with purple network of RNA.
   Normally, dogs , cats and pigs have little or reticulocytes in circulation; 0% in ungulates. Presence of reticulocytes in noticeable number in peripheral circulation is therefore a regenerative reaction of the bone marrow.

DETERMINATION OF PACKED CELL VOLUME [HAEMATOCRIT]

This is generally to determine the proportion in volume of the red blood cells corpuscles in relation to the total volume of blood in circulation.

It provides a good evaluation of the RBC status ie. information about the erythrocytes and haemoglobin in the circulatory system.this is b/c the volume of the rbc in normal blood is directly proportional to their no. and to the Hbg. Value.

General method:

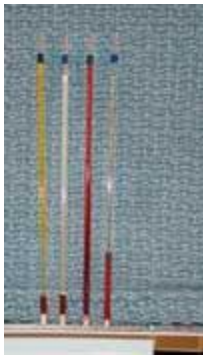
A plane capillary tube is filled with anticoagulated blood eg. Blood with EDTA. Alternatively, freshly collected blood could be filled into heparinized (heparin-coated) capillary tubes, which are available commercially. Likewise heparinized capillary tubes can be used to directly collect blood from the animal eg. from the ear vein. The capillary tubes are filled to about 👌 or 🤌 of the length. The tubes are sealed at one end either with plastiseal or the use of Bunsen burner. The tubes are arranged in a special microhaematocrit centrifuge which is fitted with a head for carrying up to 24 capillary tubes. The capillary tubes are arranged in a circular manner with the sealed end outward (centrifugal) and the open end towards the centre (centripetal). The properly covered m-centrifuge is set to rotate for 5 minutes at 12,000 rpm. PCV is read by the use of special microhaematocrit Reader. Buffy coat is a band of leucocytes and thrombocytes immediately above the packed red cells.

Inferences:

-Anaemia-------Low PCV value

-Normal PCV

-Haemoconcentration-----High PCV value



Total Red Blood Cell Count.

Because of the large population of rbc in the blood, examination is only possible after reasonable dilution has been made with suitable diluting fluid.

Eg.

a. Hayem's diluting fluid:
    -1g. of Sodium chloride
    -5g of Sodium sulphate
    -0.5g of mercuric chloride
    -200ml distilled water.
    Filter if necessary.
b. Dacies' fluid:
    -99ml of 3% aq. Solution of Sodium citrate
    -1ml of 40% formaldehyde
    Preferred for it keeps and preserves the cells better.
c. Physiological saline- where clumping occurs as a result of the above fluids.

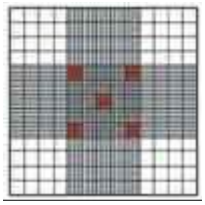
The basic equipment required in rbc total count are: microscope and haemocytometer with the diluting pipettes. Blood sample is initially drawn into the red dil. pipette held in horizontal position to the **0.5 mark** on the stem. The open end can be wiped off and can be used to withdraw the overdrawn blood by the use of cotton wool or filter paper. In a vertical position in the dil. fluid, draw the volume by gentle suction and rotation into the pipette beyond the bulb to the **101mark** . The pipette is further gently mixed for about 2 minutes with the thumb and a finger at both ends in a horizontal position.

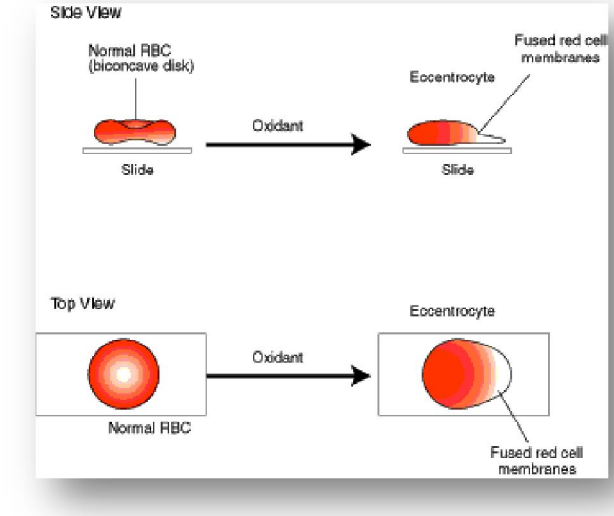
2 chambers separated by Central Moat

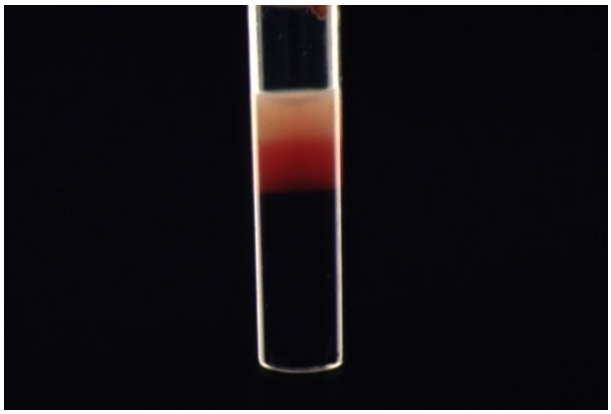
25 groups of 16 squares each =400 small squares.

It is recommended to select the 4 corner groups and the central group of 16 smallest squares, giving

1. Count only those erythrocytes which touch the left hand end and upper lines of any square
2. Disregard those touching the right-hand and bottom lines.







R=Average of two(2) fields

Area of a smallest Sq=1/400mm2
Dept =1/10mm
Volume =1/4000mm3 for a Sq.
80 Squares =80/4000=1/50mm3
Dilution factor =200
Actual =200×50×R cells
=10,000R
eg R=(661+685)÷2=673

POLYCYTHAEMIA VERA
Primary –dehydration etc
Secondary – diseases-respiratory problem
ANAEMIA
Below normal range of the RBC and or haemoglobin value
-haemorrhage
-haemolytic
-Dyshaemopoietic
-Hypoplastic
(*)-congestive heart failure
-chronic respiratory diseases
-Pulmonary &mediastinal neoplasia
There is tissue hypoxia which leads to increase in circulating RBC.

<u>Evaluation of RBC parameter</u>
Determination of Hb value:

1) Tallquist method: this is an indirect method. Comparing the colour of whole blood with a colour standard. Ther method is not sensitive and could be of upto 40% eror
   Here, a drop of blood on blotting paper is allowed to dry . the colour is compared with a colour scale graduate upto 100% = 13.8 g/100ml. the method is best used for screening purpose only
2) oxyHaemoglobin method : a direct method used to measure the oxyhaemoglobin by high absorption with the help green filter in spencer haemoglobinometer. A drop of blood is lysed in a glass chamber the

RBC are lysed by the used of applicator stick to obtain a clear fluid , which is allowed unto the haemoglobinbinometer . the Hb is read off

3) Haematin method: acid haematin or sahli method. Use of a special haemoglobinometer and pipette. Mix fresh blood with N/10HCL in a pipette to a standard level to for acid haematin. The colour is diluted to be at par with colour of a standard tinted glass within 30min the content of the pipette is there by recorded on the graduated scale.(defect in the microvasculature through which RBC circulate)
4) Cyanmethaemoglobin method: 0.02ml of blood is added to modified Drabkin's solution 9put cyanide 0.05g , K-ferrocyanide 0.20g, dist H2O TO 1L) cyanmethaemoglobin is read in a photoelectric colorimeter with suitable filter (yellow-Grreen)
5) Carboxyhaemoglobin

DETERMINATION OF MCV

Mean corpuscular volume. This is the cubical volume of the erythrocyte
MCV=(PCV×10)/RBC Fl or
PVC/RBC x 10
MEAN CORPUSCULAR HAEMOGLOBIN (MCH)
The amount of haemogobin expressed in each erythrocyte
Hb/RBC×10 pg

MEAN CORPUSCULAR HAEMOGLOBIN CONCENTRATION (MCHC)

Hb/PVC *100

CLASSIFICATION OF ANAEMIA

Base on Aetiological, morphological, responsiveness.

<u>Based on Aetiology</u>

1) Haemorrhagic anaemia
-acute blood loss (profuse blood loss eg. traumatic cut);
-chronic blood loss

*chronic haemorrhages
-GIT ulcers
-Enteritis
-Coccidiosis
-Neoplasm
-Haemophilia[dogs &foals]
-Surgical operation
-Hypersplenism

Causes

Vit C & K deficiency
Parasites eg. *Haemonchus contortus*, Hook worms, ectoparasites
Plant poisioning eg. warfarin, sweet clover, bracken fern in cattle
-Traumatic injuries
-Idiopathic thrombocytopenia purpura

2) Haemolytic anaemia- caused by lyses of RBC within the vessels
Causes-Intrinsic
-Extrinsic
Clinical Feactures:
Splenomegaly
Lymphnodepathy
Jaundice
Lethargy
Headache
A)Intrinsic causes
-RBC membrane defect eg. hereditary spherocytosis
-abnormal haemoglobinopathy eg. sickle cell
-here metabolic defect as in pyruvate kinase deficiency
B)Extrinsic causes-Acquired haemolytic anaemia eg. Idiopathic auto-immune haemolytic anaemia
-Iso-immune hereditary disorders eg. haemolytic disease of the new born.[piglets, foals, puppies,Man(children)]
-incompatible transfusion

C) Infections-eg *Babesia sp, Anaplasmosis* in ruminants, haemobatonellosis, bacillary haemoglobinuria (*Cl. haemolyticum*) in cattle & horses
D) Drugs, Chemical poisions-Cu in [sheeps], Pb, Phenothiezine, mercury in ruminants and pigs

3) Dyshaemopoietic anaemia- selective depression of erythrogenesis.
Causes :
a)nutritional deficiency –Cu , Co, Fe, Protein, Vit. Deficiency
b)parasitic diseases eg some worm infestation
c)chronic infectious diseases eg. chronic infectious nephritis in dogs, FeLV, Ehrlichiosis
d)hypothyroidism
e) Neoplasm

4) Hypoplastic (or aplastic) anaemia
Due to generalized bone marrow depression affect all other cells and platelets
This can be caused by:
-plant food poisioning eg. bracken fern in cattle, trichloro-ethylene from soya bean meal in calf
-irradiation
-sulphonamide poisoning.

Based on Morphological classification:

Based on the size of the RBC and the content of the haemoglobin eg.
a)macrocytic, normocytic ,microcytic
b)hypochromic or normochromic
combine a)&b)
-microcytic hypo chromic
-macrocytic normochromic(megaloblastic anaemia)
-macrocytic hypochromic

c) regeneration or non-regenerative anaemia

regenerative anaemia are those that the bone marrow is responding adequately(actively) by increasing the production and release of erythrocytes

into the circulation. Here one can see immature RBC in circulation[reticulocytes]. Such anaemia is characterized by the presence of polychromasia. Special stains will desmonstrate reticulocytosis.
Regenerative anaemia is a feature of haemorrhagic and haemolytic anaemias.
Non-regenerative anaemia shows that there are problems with the BM which could be either nutritional or toxic.