PRACTICAL MANUAL

OF

VETERINARY PHYSIOLOGY

(B.V.Sc. & A.H. FIRST PROFESSIONAL YEAR-(2023-24)

Volume I (Unit I-II)

NAME :----------

ROLL No. :----------

Class :----------Batch----------

DEPARTMENT OF VETERINARY PHYSIOLOGY AND BIOCHEMISTRY

MJF COLLEGE OF VETERINARY & ANIMAL SCIENCES,

CHOMU, JAIPUR (RAJASTHAN)

CERTIFICATE

This is to certify that

Mr./Ms. …… …… …… ……..……

Roll. No…… …… … ……of First year B.V.Sc& A.H. has successfully completed all practical's in Veterinary Physiology during first year of the academic year ……

……

Date :

Place :

Signature of Head of Department **Signature of Course Teacher**

FOREWORD

I am very happy to go through the Practical Manual entitled Veterinary Physiology" Department of Veterinary Physiology, MJF College of Veterinary & Animal Sciences, Chomu, Jaipur (RAJ.) The Manual covers the practical syllabus of undergraduate course (Veterinary Physiology, Volume I) Prescribed by Veterinary Council of India (New VCI 2016) for B.V.Sc& A.H. programme.

The Manual is a good attempt and is based on cumulative experience of teaching undergraduate courses. The language used in the manual is simple and lucid. the outline and description of practical exercises covering objectives, materials required, procedures and observations to be taken have been nicely presented which would be helpful in conducting practical's more effectively.

I hope this manual will make its own place in the libraries 'of Agricultural Universities, Veterinary and Animal Science Colleges and various Livestock Institutions in near future.

I congratulate the authors for the efforts put in bringing out this practical manual.

Dean
MJF College of Veterinary &
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ACKNOWLEDGEMENT

Ever since the introduction of new course for professional B. V. Sc. & A.H. degree programme under Veterinary Council of India pattern in Veterinary Colleges/Universities in the country, there was a dire need to have a practical manual on Veterinary Physiology subject which covers the practical syllabus of undergraduate (Veterinary Physiology) These new course was not dependently developed in most of the Veterinary College/University before the introduction of Veterinary Council of India programme. The present manual covers the practical with objectives, material required, procedure, steps to follow precautions to be taken, observations to be recorded and exercise to be done by the students. We hope that users will find the manual immensely useful.

We look forward to receiving the valuable suggestions of readers for improvement of this manual.

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UNIT-1

Practical No.1 **Date:**

STUDY OF MICROSCOPE

The word *micro*means small and *scope* means to see. So to see the small objects an instrument magnifies its image, called *microscope*. The magnification depends upon the system of microscope.

Principle:

The light beam, which falls on the object, passes through a series of lenses giving a clear enlarged vision of an object. There are different types of magnifying instruments as follows:

i) Compound Microscope (Monocular, Binocular, Trinocular)

ii) Dissection Microscope

iii) Transmission Electron Microscope (TEM)

iv) Phase-contrast microscope.

v) Fluorescent microscope.

vi) Scanning Electron Microscope (SEM)

vii) Interference Microscope

viii) Polarized Microscope

ix) Ultra Microscope

x) Scanning Tunneling Microscope (STM)

xi) Atomic Force Microscope (AFM)

i) Compound Microscope:

Compound microscope in commonly used in physiology laboratory.This consists of following parts:

a)**Base:**Horseshoe shaped and provides support to the microscope.

b)**Pillar:**It is the part which joins the base to main part of microscope. It has inclination joint, which helps to move the microscope in forward and backward direction.

c)**Mirror**: The mirror stand contains two mirrors on its reverse sides. One is plain mirror and other is concave mirror. These mirrors help in directing light beam towards object.

d)**Iris/Diaphragm**: It helps to regulate the amount of light.

e)**Sub stage condenser**: It condenses light on object be studied by raising or lowering it with the help of condenser screw.

f) **Stage or stage clip:** On stage, clips help to hold the slide in proper position.

g) **Stage screw:** Right side stage screw helps to move the slide in backward or forward direction, however left side screw helps to move the slide in right or left side direction.

h) **Arm:** It is used to handle the microscope. It holds the body tube that contains the objectives or eyepiece.

i) **Coarse adjustment screw:** It helps the body tube to move upward or downward direction to focus the objective accurately.

j) **Fine adjustment screw:** Helps in focusing the objective accurately.

k) **Eyepieces:** There are many different types of eye-pieces but the most generally useful are simple and comprise two Plano-convex lenses with a circular field diaphragm inter posed between them. The functions of the eye-piece are (i) The magnifications of the real image(ii) The formation of a virtual image of the real image produced by the objective(iii) To carry measuring scales, markers, cross hairs etc.

Three objectives are generally adequate for most purposes. (i) Low power (ii) High power (iii) Oil immersion

S.No.	Objectives	Eye-piece	Objective lens	Magnification
1.	Low power	10 X	10 X	100X
2.	High power	10 X	40X	400X
3.	Oil immersion	10 X	100X	1000X

PRECAUTIONS:

1. Remove all traces of ceder wood oil from the lens by a piece of flannel and xylol after use. Also clear all the lenses before and after use.
2. Do not tilt the microscope while use
3. Adjust the optical field under low power at first, then high power for details.
4. For proper focusing adjust the coarse adjustment knob/screw, then fine adjustment screw very gently.

UNIT-1

Practical No.2 **Date:**

COLLECTION AND PRESERVATION OF BLOOD SAMPLES FROM VARIOUS ANIMALS AND BIRDS

A. COLLECTION OF BLOOD:

Study of blood or hematology is an important study to know about the health status of an individual. In addition to haematological study, blood is also required for different biochemical, microbiologicalor other studies.

Different animals have different sites for collection of blood. Various sizes of needle are also required for blood collection:

Animal	Site	Standard needle gauge no.
Cattle & Horse	Jugular vein	16-18
Sheep & Goat	Jugular vein	18-20
Pig	Ear vein / Anterior vena-cava/ Tail vein	20
Dog	Cephalic / Saphenous / Jugular vein	20-22
Cat	Ear / Cephalic / Jugular vein	20-25
Rabbit	Ear vein / Cardiac puncture	18-20
Rat & Mouse	Cutting of tail / Cardiac puncture	22-25
Bird	Wing vein	20-25

Precautions:

1. Needle and syringe must be sterilized properly and dried before collection. Water or any hypotonic moisture may rupture RBC.
2. Standard needle gauge no. is to be used for a particular species.
3. Blood should not be handled roughly as RBC may rupture.

(B) PRESERVATION OF BLOOD

To keep the whole blood in liquid state some preservatives or anticoagulants are generally used in laboratory.

Anticoagulants	Quantity require	Use	Disadvantages	Mechanism of action
Heparin(natural anti -coagulant)	0.1-0.2mg/ml of blood or 10-20 I.U/ml of blood	Has least effect on size and haemolysis. The excess heparin does not change PCV, MCV, MCH and MCHC. Hence preferred in these estimates. Used for blood gas analysis and for blood Ca^{++} estimation.	It cannot be used for blood morphology as it interferes with the staining of cells (WBC). Not suitable for Prothrombin time tests and for agglutination since heparin causes clumping of WBCs.	Anti-thrombin and antithrombopla stin inhibits activation of Factor IX
EDTA (Na^+ or K^+ salts of Ethylene diamine tetra acetic acid)	**1-2mg per ml ofblood.**	Recommended for morphological studies of blood	Excessive EDTA (>2mg/ml) shrinks cells and there by effects	Acts as chelating agent. Combines with

		(R.B.C., W.B.C., Thrombocyte count, Differential count of Leucocytes, Hb estimation, PCV, Urea, N_2, Glucose, Proteins, fibrinogen content of plasma.	PCV, MCV, MCH and MCHC.	Ca^{++} to form an insoluble salt
<u>Oxalates</u> a) Potassium b) Ammonium	4 mg of (a) and 6 mg of (b) combined for 8-10 ml of blood	Suitable for routine blood studies	Not suitable for blood transfusion, NPN and BUN	Precipitate calcium as salt
Trisodium citrate (3.8% solution)	2.5 ml/1oml blood solution to be dried in sample test tube	Mainly used in blood transfusion		Precipitate calcium as salt
<u>Fluoride and Thymol</u> a) Sodium Fluoride b) Thymol	10 mg of (a) amd 1 mg of (b) combined / ml of blood	Blood glucose estimation	As it is enzyme killer it cannot be used in other estimation	Precipitate calcium as salt

UNIT-1

Practical No.3 **Date:**

SEPARATION OF PLASMA, SERUMANDPREPARATION OF DEFIBRINATED BLOOD

Preparation and separation of plasma:

The fluid portion of the blood in which all the cells remain suspended is known as plasma.

Procedure:

1. Collection of blood sample is done as in the previous exercise.

2. About 10ml of freshly collected blood sample containing anticoagulant is taken in a clean, dry and sterile test tube.

3. After that it is centrifuged at 3000 rpm for 30 minute so that cells settle down as they are heavier than plasma.

4. After centrifugation collect the supernatant with pipette into another test tube or vial and store in refrigerator.

Preparation and separation of serum:

Procedure:

1. Blood collection is done as in the previous exercise.

2. Freshly collected blood sample is taken into a clean, dry and sterile test tube without adding any anticoagulant and then place the tube in slanting position for 10-15 minutes till blood is clotted.

3. After that tube is kept in incubator at 37^{o}C for 1-2 hours without any shaking.

4. After clot formation, remove the clot by centrifuging the sample test tube at 3000 rpm for 30 minutes in a refrigerated centrifuge.

5. The resultant supernatant is called as serum.

6. Following centrifugation transfer the serum (liquid component) into a clean polypropylene tube using a pasture pipette.

7. Sample should be maintained at 2-8°C while handling and stored and transported at -20°C.

Preservation of serum:

The most common method of serum preservation is freezing at -20^{o}C (deep freeze) or below. When serum is to be stored for more than a few days the preservation of serum can be achieved by:

(1) Preservatives: eg. 0.0001% Merthiolate, 0.25% Phenol & 0.20% Sodium azide

(2) Sterilization: By filtration through

(i) Seitz filter for large quantities

(ii) Millipore filters (0.22-0.45 µ pore size) for small quantities.

(3) Lyophilization: In this process the water is extracted by vacuum while the serum is kept cold by a dry ice – alcohol mixture. The tubes containing the cold dried serum are sealed and stored at cold temperatures.

Procedure of preparation of defibrinated blood:

1. The defibrinated blood is composed of serum in which red and white blood cells remain suspended and this blood may be used for separation of serum, isolation of leukocytes and collection of erythrocytes for osmotic fragility test.

2. Collection of blood is done as in the previous exercise.

3. About 10 ml of freshly collected blood sample is taken into a suitable container or conical flask of 50 ml capacity without adding any anticoagulant.

4. It is constantly rotated with the help of a glass rod or glass beads for about 10 minutes.

5. In this way all the fibrinogen is converted into fibrin within a short time and fibrin clot sticking to the glass rod or beads is removed from the sample.

6. This blood will not clot any further and remains fluid. This blood is now known as defibrinated blood.

UNIT-1

Practical No.4 **Date:**

DETERMINATION OF CLOTTING TIME OR COAGULATION TIME.

Principle:

The coagulation time of blood is the time required for venous blood to clot after it has come out of the vessel. The final step of blood clotting process results in the formation of fibrin threads from fibrinogen present in the blood plasma. The formed fibrin threads hang the small bit of broken capillary tube.

Capillary method*:*

Apparatus: Stop watch, Spirit, Cotton, Sterilized lancet, Capillary tube.

Procedure:

- ❖ Sterilize the area where you are going to prick.
- ❖ Prick the fingertip or ear vein. Start the stopwatch when the puncture is made.
- ❖ Immediately apply the tip of the capillary tube to the drop of blood at an angle. By capillary attraction, the capillary tube is filled.
- ❖ At every 15 seconds interval, with the help of forceps break the capillary tube bit by bit.
- ❖ Stop the watch when a fibrin thread is formed between the two broken pieces allowing the broken piece to hang on to the other piece of the capillary tube. This duration is taken as clotting time.

Normal blood clotting time (minutes):

Animal	Capillary tube method
Cattle	3.0 – 15.0
Horse	4.0 - 15.0
Sheep	1.0 - 6.0
Goat	3.0- - 12.0
Pig	2.5 - 4.0
Dog	3.0 - 13.0
Cat	4.5 - 8.0
Human	4.0 - 10.0

UNIT-1

Practical No.5 **Date:**

DETERMINATION OF BLEEDING TIME

It is the time taken for a small, standardized wound to stop bleeding.

Principle:

The bleeding from the blood capillaries cease after the formation of platelet plugs at the cut ends of capillaries.

Duke's method:

Apparatus:

Spirit, Lancet, Blotting paper, Stopwatch

Procedure:

- An ear lobe or tip of the finger is punctured deeply with a sharp sterile lancet.
- Start the stopwatch as soon as drop of the blood comes out.
- Gently go on blotting the blood at 30 seconds interval with the dried and cleaned filter or blotting paper without pressing.
- The size of the blood spot on blotting paper goes on getting reduced and gradually disappears.
- Stop the watch when bleeding stops and blood drop does not stain the filter paper time. Take this duration as bleeding

  Normal range ------- 1-10 minutes.

  Average duration----2-3 minutes.

  Reading above 10 minutes is abnormal.

  Human ---------- 2.5 minutes.

This method is not accurate because of variability of test conditions like depth of puncture, puncture site, amount of hydrostatic pressure within the capillaries etc.

UNIT-1

Practical No.6 **Date:**

ESTIMATION OF HAEMOGLOBIN CONCENTRATION

Objective:

Estimation of haemoglobin content of blood by Sahli'shaemoglobinometer method.

Principle:

Hemoglobin in the blood is converted into acid haematin with the addition of dilute hydrochloric acid. The colour so formed is, compared with the coloured glass standard to obtain the haemoglobin concentration in the given sample.

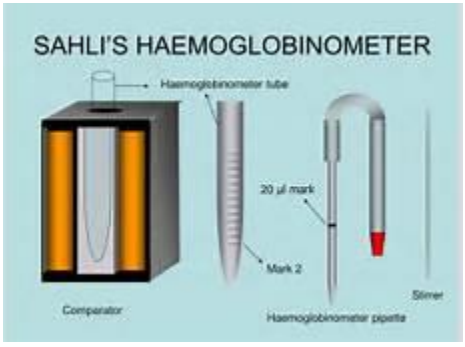
Apparatus/Material:

1. **Hellige-Sahli'shaemoglobinometer set** consists of a comparator, a graduated tube, a micropipette, and a glass rod (stirrer). The comparator contains a two colored standard glass prisms to compare colour of acid haematin. Haemaglobinometer tube is a long tube having divisions on two sides. On one side denoting the concentration of haemoglobin in gm% and on the other side the percent haemoglobin content (%). Haemoglobinometer pipette has got a mark of 20cmm or 20µl.

2. N/10 HCl
3. Distilled water
4. Dropper
5. Blood sample.

Procedure:

- In a graduated haemoglobinometer tube, take N/10 HCl up to the mark 2.
- Draw the thoroughly mixed blood up to the 20µl mark in haemoglobinometer pipette. Wipe the tip and carefully discharge the blood in to the tube containing N/10 HCl.
- Blood and acid in the tube are thoroughly mixed with the help of a glass rod (stirrer) for about a minute until the mixture gives uniform dark brown colour.
- The tube is allowed to stand for 10-15 minutes (reaction period) for complete conversion of haemoglobin in to acid haematin.
- The mixture is then diluted with distilled water. Distilled water is added drop by drop, every time mixing with the stirrer.
- Remove the stirrer and compare the colour of the mixture with the colored standard rods on either side.



- ❖ When the colors match, stirrer is taken out from the diluting tube taking care not to allow a drop of solution to be carried away with the stirrer. Read the height of the mixture in graduated tube in terms of percentage or grams percent from the graduations on the other side of the tube.
- ❖ 14.4 gm% of haemoglobin is equal to 100% Sahli's value.

Precautions:

- ❖ Hold the instrument between your eye and day light to avoid error.
- ❖ While matching colour, hold stirrer above the level of mixture but do not remove the stirrer entirely from the measuring tube as this may result in loss of solution adhering to the stirrer.
- ❖ While matching colour, blank portion of diluting tube should face the observer but not graduated portion.

The mixture should appear clear (without blood clots) and brown in colour**.**

Interpretation:

1. Increase in the hemoglobin conc. Can be occurring in the following conditions.

i. Dehydration ii. Haemoconcentration iii. At high altitude iv. At birth

v. Chronic CO poisoning vi. Emphysema

2. Decrease in the hemoglobin conc. Can be occurring in the following conditions.

i. Anaemia ii. Haemorrhage iii. Lead poisoning iv. Leukemia

v. Pneumonia vi. After fluid therapy vii.Leishmaniasis viii. Nephritis

OBSERVATION:

UNIT-1

Practical No.7 **Date:**

DETERMINATION OF PACKED CELL VOLUME OR HAEMATOCRIT VALUE.

Packed Cell Volume is the ratio of volume of erythrocytes to that of the whole blood expressed as percentage.

Principle:

The blood cells are force into a packed column by the centrifugal force when subjected to the centrifugation in a special tube. Heavier red cells settle at the bottom with white cells and platelets forming a layer over the R.B.C. column.

Wintrobe's method (Macro-method):

Apparatus/ Material required:

1) Wintrobehaematocrit tube: 3mm bore and 110mm height calibrated to 100mm with 1mm intervals. The numbers on the right start with zero from the bottom to 10 cm at the top. The reverse marking on the other side of the tube with zero at the top and 100 at the bottom. It accommodates about 1 ml of blood.

2) Syringe with a long spinal needle.

3) Centrifuge machine.

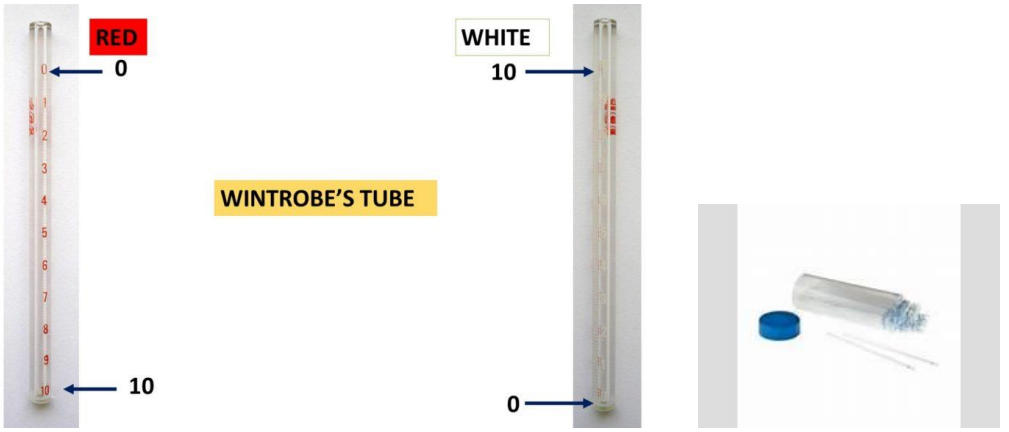
4) Fresh blood mixed with anticoagulant.

Procedure:

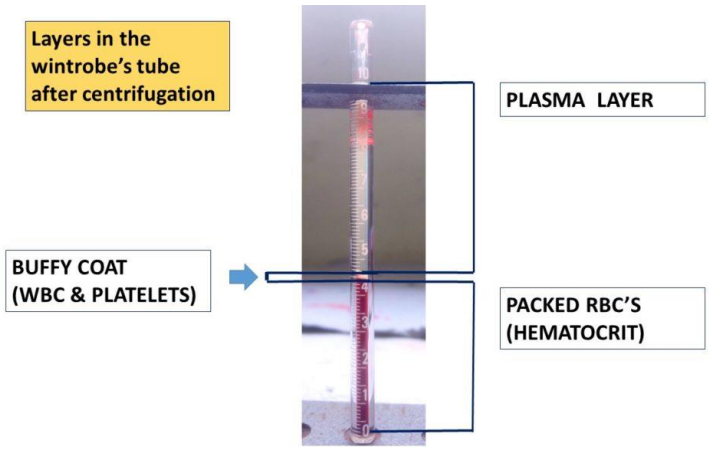
- ❖ Mix the blood sample thoroughly by repeated inversion of the vial.
- ❖ Take the blood in a syringe without air bubble and introduce long needle to the bottom of the tube. Fill the haematocrit tube by gently pressing piston of the syringe. As the Wintrobe is filled from bottom to top, the long needle attached to the syringe is slowly withdrawn, keeping the tip of needle all the time just below the surface level of blood as it is filled in the tube. Fill the Wintrobe tube up to 100 marks. Use duplicate samples.
- ❖ Centrifuge at 3000 rpm for 30 minutes. After centrifugation take out the tubes and read the cellular layers as follows:
- ❖ Bottom of the tube contains dark brown column of red cells, which is read as PCV.

❖ Above the dark brown column of red cells is a buffy coat consists of grayish layer of leucocytes. In between the layers of leucocytes and plasma, a thin creamy layer of platelets can be seen.

❖ Express the PCV as a percentage of total blood columns taken.



CAPILLARY TUBE



Observation:

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UNIT-1

Practical No.8 **Date:**

DETERMINATION OF ERYTHROCYTE SEDIMENTATION RATE (ESR)

Objective:

Determination of Erythrocyte Sedimentation Rate (ESR).

Principle:

Blood cells tend to settle down when anticoagulated blood is kept undisturbed in vertical position, because of their higher specific gravity compared to that of plasma. This process is called sedimentation and the rate of settling down or sedimentation is known as Erythrocyte Sedimentation Rate.

ESR is performed as an aid to diagnosis and evaluation of disease process. In general, ESR increases in all inflammatory diseases of animals except horse. ESR is of negligible importance in cattle, sheep and goat. Among animals, ESR is of value only in dog. ESR depends on the concentration of macromolecules in plasma, especially fibrinogen. ESR is faster with macromolecules present.

ESR depends on:

- Differences in densities between RBC and plasma.
- Degree of adherence of RBC to one other (rouleaux formation) related to the plasma protein content and on the resistance that plasma exerts on the red cell surface.
- RBC carries a negative charge, and hence any condition that increases the positive charge accelerates the process.

Westergren's method:

Apparatus

1. Westergren pipette – It is a 300mm long uniform bore glass tube of 3mm internal diameter and graduated from zero to 200mm at 1mm interval. Each tube holds about 2ml of blood.
2. Citrated blood – Add 1ml of 3.8% sodium citrate for 4 ml of blood.

Procedure:

- Mix the citrated blood thoroughly without any froth.
- Suck the citrated blood in to the Westergren pipette up to zero mark.
- Fix the pipette in the ESR stand in between the rubber corks at the sole and spring pads at the top. The pipette should be in vertical position. Observation is taken by noting the point on the mm scale to which the erythrocytes fall in exactly 10 minutes. Keep on recording the sedimentation at 15 minutes interval. Take the final reading of cell columns after 1 hour. The difference between final and initial readings gives the sedimentation rate per hour.
- Compare the observed ESR with the anticipated ESR for the PCV of that blood sample. The difference between the observed and anticipated rates is

called the corrected ESR. The corrected ESR should be used to evaluate the influence of disease.

OBSERVATION:

Initial reading =

Final reading after 30 minutes=

Final reading after 60 minutes =

Final reading after 2 – 3 hours=

Calculation and results:

ESR (mm/hour) = Final reading – Initial reading

Interpretation:

1. ESR value may be increased in the following conditions.

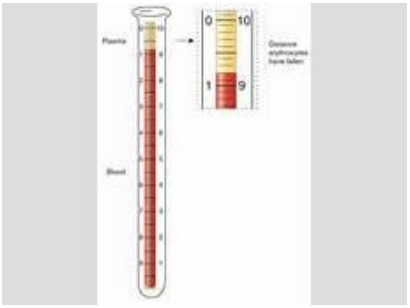
i. Anaemia ii. Pregnancy iii. Rehydration iv. Inflammation v. Neoplasia

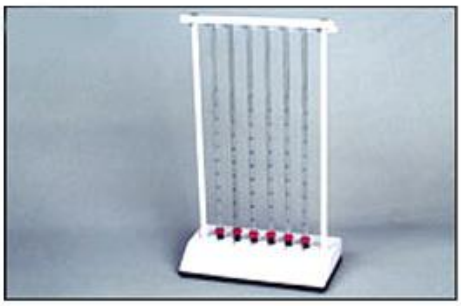
vi. Acute generalized infections vii. Chronic localized infections viii. Hypothyrodism

2. ESR value may be decreased during dehydration.

Rouleaux formation:

RBCs carry negative charge which results in repulsion. Due to repulsion the RBCs remain separated from each other in plasma but the plasma proteins impart cohesive property over repulsion. During movement RBCs strike each other, tend to stick to each other and pile over one another to form rouleaux (about 8-10 cells). The density of the mass now increases and rouleaux sinks down quickly. The factors affecting rouleaux formation will also affect the ESR. Protein and cholesterol in plasma and increased number of RBCs will increase the rouleaux formation.





Observation:

Practical No.9 **Date:**

ENUMERATION OF ERYTHROCYTES

Objective: Determination of erythrocytes number in the given blood sample.

Principle:

A small, measured quantity of blood is diluted with a suitable extender i.e. diluting fluid, which will prevent haemolysis of erythrocytes. Small drop of this mixture is placed on counting chamber of haemocytometer and the number of erythrocytes are counted in a known area under a high power of microscope and then calculated in to the number of erythrocytes per cu.mm. of blood, taking the dilution factor into consideration.

Apparatus/Materials:

1) Blood sample (2) Erythrocyte diluting fluid

3) Compound microscope (4) Haemocytometer with mechanical stage.

Haemocytometer:

It consists of:

1. Diluting pipette for R.B.C.
2. Diluting pipette for W.B.C.
3. Thick glass slide with 2 improved Neubauer counting chambers
4. Square cover slip
5. Rubber tubes with mouthpiece.

The diluting pipette consists of a bulb and a stem. The bulb of R.B.C. diluting pipette has a red bead and W.B.C. diluting pipette has a white bead to assist mixing of blood and diluting fluid. The long stem portion below the bulb has 2 markings with a value of 0.5 given to the first and that of 1 given to the 2^{nd} marking. Above the bulb on short stem portion the R.B.C. pipette has a mark of 101 and correspondingly 11 in W.B.C. diluting pipette.

Neubauer Counting Chamber*:*

The thick glass slide is divided into three platforms, two lateral ones and one central with the help of trenches. The central platform is further divided into two equal halves with the help of a trench, giving H shaped appearance. The central platform is 1/10mm lower (depth) than the platform on either side. Each sub division of the central platform has improved Neubauer counting chamber for counting of red

and white cells. Each Neubauer counting chamber consists of 9 sq.mm. Area (3 mm x 3mm) divided into 9 big/primary squares of 1sq.m.m. The central square of 1sq m.m. area is the RBC counting chamber. The four corner squares are used for the enumeration of WBCs. The RBC counting chamber is divided into 25 medium/secondary squares separated from each other with the double lines, each. Further, each secondary square is subdivided into 16 small/ tertiary squares bounded by triple lines i.e., the central square is divided into 400 small squares (25x16).

Diluting fluid:

Many varieties of fluids are used for extending or diluting the blood. The diluting fluid should prevent clotting, rouleaux formation or agglutination of blood cells. It should be isotonic to the blood, and should contain a fixative to preserve the shape of RBCs to prevent autolysis of the cells. It should have good keeping quality, preventing growth of bacteria, molds and fungi. The composition of most commonly used diluting fluids is as follows:

(I) **Hayem's fluid:**

Mercuric chloride	---	0.25gm
Sodium chloride	---	0.50gm
Sodium Sulphate	---	2.50gm
Distilled water	---	100ml

(II) **Gower's fluid:**

Sodium Sulphate	---	12.5gm
Glacial acetic acid	---	33.3ml

Distilled water added up to 200ml.

Procedure:

- Check the R.B.C. pipette and see that it is completely dry and clean.
- Clean and dry the glass slide and cover slip.
- Place the cover slip over the central platform of glass slide. The cover slip rests on the ridges found on either side of the central platform.
- Mount the slide on the mechanical stage of the microscope.
- Focus the counting chamber by using the low power of the microscope.
- Draw the uncoagulated blood up to 0.5 mark of R.B.C. diluting pipette.
- The blood sample should be shaken well before taking blood into the pipette. If the blood is drawn more than the required, blood can be adjusted back to the 0.5 mark by tapping the tip of pipette with the tip of the finger. Never use

cloth or blotting paper for this purpose as it tends to withdraw the fluid portion of the blood leaving a higher concentration of cells.

- Suck the diluting fluid up to 101 mark while doing so avoid air bubbles.
- Mix the blood by holding pipette in between thumb and index finger.
- Discard 3 or 4 drops of unmixed diluting fluid from the stem of the pipette this is done because in the stem portion (upto 1 mark) there is no mixing of blood with the diluting fluid thus the total dilution(dilution factor) of the blood is 200.
- Hold the pipette at 45 º angles to the surface of counting chamber and place the tip to the narrow slit between the counting chamber and the cover slip. The fluid spreads between cover slip and counting platform by capillary action. Avoid air bubbles. Do not allow the fluid to over flow on the cover slip and into the grooves of counting chamber. If the fluid overflows the whole procedure has to be repeated.
- Allow the cells to settle down for about 1-2 minutes.
- Focus the chamber under low power, then to high power and make the count as follows.

Counting of RBC

For accurate results counting has to be done in the entire R.B.C. square. But for routine work, to facilitate easy counting of R.B.C, the cells over 5 medium squares (top left, top right, bottom left, bottom right and central) can be counted under 40X (high power). While counting, if the cells touching the left and upper boarder lines of squares are included, ignore the cells touching right and bottom boarder lines. If the difference between the counts of any two squares exceeds 10% of average count, it indicates uneven distribution of cells over the counting chamber. In such circumstances chamber is to be cleaned and recharged with the extended blood.

Calculation:

Let, N = the number of cells counted in 80 small squares.

Then, N/80 = the number of cells in 1 small squares.

The area of 1 small square = 1/400 sq mm.
and the depth = 1/10 mm.

Volume of 1 small square = 1/400 x 1/10 = 1/4000 cumm

Then, result is given as the number of cells in 1 cubic m.m.

Therefore, N/80 multiplied by 4000.

The blood was originally diluted 1 in 200.

Therefore, No. of red cell/ cu m.m. $= \frac{N \times 4000 \times 200}{80}$

$= N \times 10000 = N \times 10^4$

Results:

Express the result in **millions of R.B.C. per cu.mm of blood**. Compare result with normal values of the given species of animal and give comments as normal, anaemic or polycythemic.

Precautions:

- Use clean and dry glassware.
- Pipette should be checked for blockage or a broken tip.
- Take exact quantity of blood thoroughly. If the blood is drawn more than the required, blood can be adjusted back to the 0.5 mark by stroking the tip of pipette with the tip of the finger. Never use cloth or blotting paper for this purpose as it tends to with draw the fluid portion of the blood leaving a higher concentration of cells.
- In case of anaemia it is better to take blood up to the mark 1.0 to improve the accuracy of results.
- Mix well the blood and diluents.
- Avoid air bubbles in the counting chamber.
- Avoid under or over filling of the counting chamber.

Interpretation:

1. Polycythemia : It is a condition in which the number of red cell count is increased than the normal level. It can occur in the following conditions.

(A) Physiological conditions:

i. At high altitude ii. New borne animals iii. Dehydration iv. Stress

v. Lowered O_2tension vi. Epinephrine injection

(B) Pathological conditions :

i. Acidosis ii. Diarrhoea iii. CO poisoning iv. Vomiting v. Emphysema

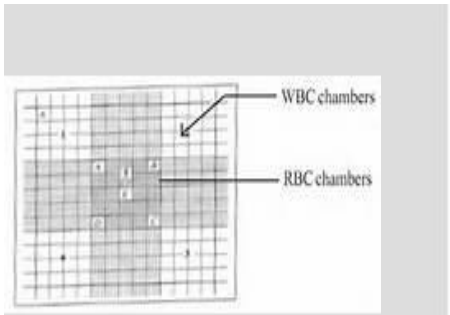
Vi. Haemoconcentration vii. Chronic heart disease viii. Nephritis

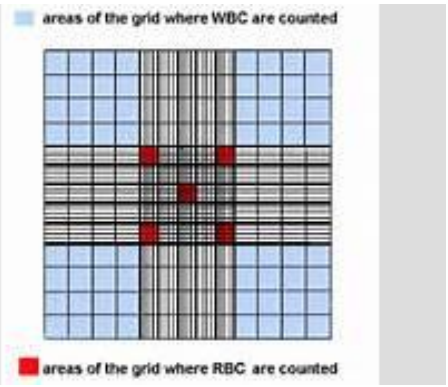
ix. Bronchitis

2. Oligocythemia : It is a condition in which the number of red cell count is decreased

than the normal level. It can occur in the following conditions.

i. Injection of fluids ii. Anaemia iii. Hemorrhage iv. Leukemia





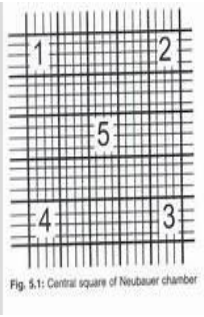


Fig. 5.1: Central square of Neubauer chamber

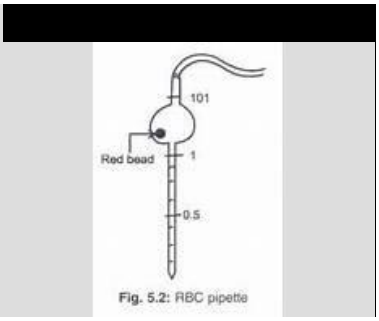


Fig. 5.2: RBC pipette

OBSERVATION & RESULT:

Hematological indices (or) Absolute Erythrocyte Corpuscular indices.

On the basis of erythrocyte count, haemoglobin concentration and packed cell volume the characteristics of individual red cells (i.e. red cell indices) can be formulated. The Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) have been referred to as absolute values (or) red cell indices. These indices are useful in diagnosis various types of anaemias.

Mean corpuscular volume (MCV):

It is the volume of the average red cell in a given blood sample. It is calculated as follows:

$$\text{MCV (fl or femitolitre)} = \frac{\text{PCV (\%)} \times 10}{\text{RBC number (millions /mm}^3\text{)}}$$

Mean corpuscular haemoglobin (MCH):

It is the amount of haemoglobin by weight in the average red cell of the blood sample. It is calculated as follows:

$$\text{MCH (Pico grams)} = \frac{\text{Haemoglobin (gm \%)} \times 10}{\text{RBC number (millions/mm}^3\text{)}}$$

Mean corpuscular haemoglobin concentration (MCHC):

It is the concentration of haemoglobin (weight per volume) in average red cell of the blood sample. It is calculated as follows:

$$\text{MCHC (\%or gm/dl)} = \frac{\text{Haemoglobin (gm \%)} \times 10}{\text{PCV (\%)}}$$

Microcytosis – MCV lower than normal (90 ± 8fl)
Macrocytosis—MCV higher than normal
Hypochromia –MCHC lower than normal
Hyperchromia- MCHC higher than normal.

Normal hematological values in **human beings**:

MCV ----- 90 ± 8 fl
MCH ----- 30 ± 4 pg
MCHC -- 34%

OBSERVATION:

UNIT-1

Practical No.10 **Date:**

ENUMERATION OF LEUCOCYTES

Objective:

Enumeration of leucocytes in the given blood sample

Principle:

A small, measured quantity of blood is diluted with a suitable extender i.e. diluting fluids, which will haemolyse only the erythrocytes but leave the leucocytes intact. Small drop of this mixture is placed on counting chamber of haemocytometer and the numbers of leucocytes are counted in a known area and then calculate in to the number of leucocytes per cu.m.m. of blood.

Apparatus/Materials:

1. Blood sample
2. Leucocytes diluting fluid
3. Compound microscope with mechanical stage
4. W.B.C. diluting pipette
5. Improved Neubauer counting chamber
6. Cover slip.

Composition of W.B.C., diluting fluids:

I) Thomas W.B.C. diluting fluid:

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

Procedure:

- Check the W.B.C. pipette and see that it is completely dry and clean.
- Clean and dry the glass slide and cover slip.
- Place the cover slip over the central platform of glass slide. The cover slip rests on the ridges found on either side of the central platform.
- Mount the slide on the mechanical stage of the microscope.
- Focus the counting chamber by using the low power of the microscope.
- Draw the uncoagulated blood up to 0.5 mark of W.B.C. diluting pipette.
- The blood sample should be shaken well before taking blood in to the pipette. If the blood is drawn more than the required, blood can be adjusted back to the 0.5 mark by tapping the tip of pipette with the tip of the finger. Never use cloth or blotting paper for this purpose as it tends to withdrawn the fluid portion of the blood leaving a higher concentration of cells.

- Suck the diluting fluid up to 11 marks to get a dilution of 1:20 in the mixing chamber. While doing so avoid air bubbles.
- Mix the blood by rotating pipette in between thumb and index finger or in between palms.
- Discard 3 or 4 drops of unmixed diluting fluid from the stem of the pipette.
- Hold the pipette at 45 º angles to the surface of counting chamber and place the tip to the narrow slit between the counting chamber and the cover slip. The fluid spreads between cover slip and counting platform by capillary action. Avoid air bubbles. Do not allow the fluid to over flow on the cover slip and into the grooves of counting chamber. If the fluid overflows the whole procedure has to be repeated.
- Allow the cells to settle down for about 1-2 minutes and count the number of W.B.C. in four large (1mm) corner squares of counting chamber under low power of microscope with the condenser lowered. Adjust the light so that leucocytes appear as round, slightly indented bodies with a definite outline. If there is a doubt as to whether the objective seen is leucocytes or an artifact examine it under high power magnification. Leucocytes have definite cell outlines and well defined nuclei. Platelets appear as very small refractile bodies about $1/5^{th}$ to $1/7^{th}$ size of leucocytes without nuclei.

Calculation:

- Let the total number of W.B.C. present in four squares of 1sq.mm area of 1/10 mm depth i.e., 4 x 0.1 cu mm area = $A_1 + A_2 + A_3 + A_4 = X$
- The number of W.B.C. present in 1 sq. mm area of 0.1 mm depth i.e., in 0.1 cu.mm. Volume = X/4.
- The number of W.B.C. present in 1 cu. mm volume = x/4 x 10
- The original blood sample is diluted 20 times i.e., 0.5 parts in 10(11-1) parts.

Therefore 1 cu.mm.of undiluted blood contains X/4 x 10 x 20 i.e. X x 50

Interpretation :

1. Leukocytosis : It is the increased WBC count than the normal level and can be occur in the following conditions.

(A) Pathological conditions

i. Leukemia ii. Hemorrhage iii. Diabetes iv. Neoplasm

v. Acute bacterial infections vi. Parasitic infestations

(B) Physiological conditions

i. Dehydration ii. Pregnancy iii. Exercise iv. New born animals

v. Estrus

vi. Fear or excitement

2. Leukopenia : When WBC count is decreased than the normal value and it can occur

in the following conditions.

(A) Pathological conditions

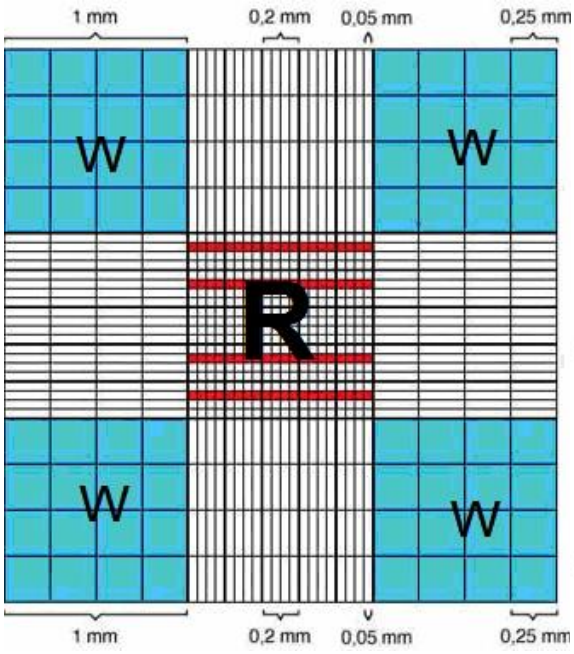
i. Anaemia ii. Pellagra iii. Anaphylactic shock iv. Viral infections

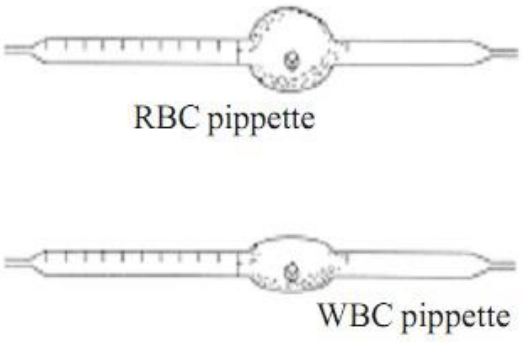
v. Myxoedema vi. Food allergy vii.Analgesics viii. Corticoids

ix. X-ray exposure

(B) Physiological conditions

i. After fluid therapy





OBSERVATION & RESULT:

UNIT-1

Practical No.11 **Date:**

DIFFERENTIAL COUNT OF LEUCOCYTES (DLC)

DLC – It may be defined as number of various types of leucocytes per hundred WBC counted.

Objective: To identify and count different types of leucocytes

Principle:

The stained white cells can be differentiated into many types depending on their physical cell characteristics like size and shape of the cell; size, shape and location of the nucleus; position, size, shape and colour of the cytoplasmic granules and appearance of cytoplasm.

Apparatus required:

1) Microscope with oil immersion objective and mechanical stage.

2) Clean, grease free, dry glass slides.

3) Staining rack.

4) Cedar wood oil.

5) Phosphate buffer (0.01 M, pH6.8) (or) Distilled water.

6) Staining solution: Leishman's /Wright's / Giemsa's stain.

7) Blood sample

Leishman's stain:

1. Leishman's powder (Methylene blue and Eosin): 0.15gm
2. Acetone free Methanol: 100ml

Preparations of blood smear:

- Place a small drop of fresh or anticoagulant mixed blood on clean, grease free, dry glass slide about 2cm away from one end.
- Place another slide i.e. spreader slide in front of the blood drop. Make the angle between two slides 30º – 45º. Greater the angle would be the smear.
- Draw the spreader slide backward till it touches the drop of blood. Let the blood spread across the surface between two slides.
- With a single swift forward movement of spreader slide, spread the blood along the surface of slide.

- Dry the smear immediately by waving the slide rapidly or exposing it to the air. The rapid drying of smear prevents crenation of cells.
- The dried smears can be stained on a staining rack.
- Staining of blood smear with Leishman's stain or Wright's stain:
- Place the slide on staining rack.
- Cover entire blood smear with Leishman's/Wright's stain and allow it to act for 1-2 minutes at room temperature.
- Add equal quantity of distilled water or phosphate buffer and thoroughly mix it with the stain either by rocking movement or blowing air through a glass tube.
- Incubate it for 5-10 minutes at room temperature. If room temperature is very low proportionately increase the incubation time.
- Wash off the stain solution gently with distilled water.
- Dry the smear in the air and focus under high power to see the distribution of cells and nature of staining.

Staining the blood smear with Giemsa's stain:

- Keep the slide on staining rack and pour 5 drops of Methyl alcohol on the blood smear to fix the smear.
- Let the methyl alcohol to act on the smear for 1-2 minutes.
- Then wash the slide under tap water. Place the washed slide on staining rack again and pour the Giemsa's stain on it and allow it to act on smear for 40-45 minutes.
- During this period don't allow the stain to dry. After 45 minutes wash the slide dry in the air and then examine under microscope.

Counting of different Leucocytes:

Place a small drop of cedar wood oil on blood smear and focus it under oil immersion lens.

When a smear is made there is tendency for neutrophils to rush to the edges, the lymphocytes are concentrated in the body of smear, while the monocytes and eosinophils are evenly distributed throughout the smear. Therefore for counting it is better to follow cross sectional method which consists of going back and forth across the slide. Count minimum one hundred (better 200) white blood cells.

Identification of cells:

Romanowsky stains are used for routine examination of blood smear. The Romanowsky stains used are Jenner's stain. Leishman's stain, Wright's stain, Giemsa's stain. The Azure B (Trimethylthionin) in Romanowsky stains is a basic dye. Basophilic granules contain heparin, which has an affinity for basophilic dye and stains deep blue. Eosinophilic granules contain a spermine derivative with an alkaline grouping, which stains strongly with acidic components of the dye i.e. Eosin Y (Tetrabromoflorescein) giving red colour to the granules. Granules of neutrophils are weakly stained by Azure complex and stains violet i.e. a mixture of red and blue colour.

Precautions:

- ❖ The blood smear should not extend to the edges of the glass slide.
- ❖ Smear should not be too thick or too thin.
- ❖ Do not allow the stain to dry before it is diluted, to prevent the precipitation of stain particles.
- ❖ Do not wash the slides for long periods, which will wash away the stain from the stained cells.
- ❖ Washing water or the buffer should not be too alkaline, as the cytoplasm of leucocytes will not be stained.
- ❖ Washing water or the buffer should not be too acidic, as the nuclei of leucocytes will not be stained.

OBSERVATION & RESULT:

UNIT-1

Practical No.12 **Date:**

ENUMERATION OF THROMBOCYTES

Objective:

Enumeration of thrombocytes in the given blood sample.

Principle:

A small, measured quantity of diluted with a suitable extender i.e., diluting fluid, which selectively stains thrombocytes enabling easy identification and counting. Small drop of this mixture is placed on the counting chamber of haemocytometer and the number of thrombocytes is counted in a known area and from that number of thrombocytes present in undiluted blood sample can be computed.

Apparatus/Materials:

(1) Blood sample. (2) Thrombocyte diluting fluid.

(3) Compound microscope. (4) R.B.C. diluting fluid.

(5) Improved Neubauer counting chamber. (6) Cover slip.

(7) Petri dish. (8) Filter paper.

Composition of various Platelet diluting fluids is as follows:

i) Rees-Ecker's fluid:

Sodium citrate --------- 3.8 gm
Formaldehyde (40%) - 0.2 ml
Brilliant cresyl blue---- 0.1 gm
Distilled water --------- 100 ml

ii) Solution-I:

2% EDTA in 85% NaCl. Add brilliant cresyl blue 2 crystals.
Filter the diluting fluid before use.

iii) Solution-II:

Ammonium oxalate ---- 1 gm
Brilliant cresyl blue ---- 0.05gm
Distilled water ----- 100 ml

Procedure:

- Wash the R.B.C. diluting pipette by taking the pre-filtered diluting fluid up to 1 mark and expel it immediately in order to moisten the wall of the pipette and thereby to avoid adherence of thrombocytes to the pipette.
- Draw EDTA mixed blood up to the mark and dilute it up to 101 mark with diluting fluid.

- Mix the contents of pipette thoroughly for five minutes.
- Discard first four drops of the fluid from the pipette and charge both the counting chambers of haemocytometer.
- Allow 15 minutes for the platelets to settle down in the counting chambers. During this period of settlement place the haemocytometer in a Petri dish having moistened filter paper. Care should be taken to keep haemocytometer slide away from the moistened filter paper by resting the slide up on to two applicator sticks or on rubber caps of glass vials. The purpose of keeping haemocytometer slide in the Petri dish is to prevent evaporation of the fluid that is charged on the counting chambers.
- Focus the R.B.C. square using high power objective under dim light. Count the thrombocytes (oval, round or comma shaped, purple or pink purple) present in both the R.B.C. squares (of each 1sq.mm area) on both the sides.

Calculation:

- One side area of R.B.C. square is 1sq.mm. Since the thrombocyte counting is done on both sides, the total area for thrombocyte count is 2sq.mm. and volume is 0.2 cu. mm (2sq.mm x 0.1 mm depth).
- Let the number of thrombocytes present in 0. 2 cu. mm of diluted blood = Y
- The number of thrombocytes present in 1 cu.mm of diluted blood = Yx 5 .Dilution factor is 200.
- The total number of thrombocytes present in 1 cu.mm of undiluted blood is Y x 5 x 200 i.e., Y x 1,000.

Calculation:

No. thrombocytes / microlitre = No. of thrombocyte x 1000

Exercise: Estimation of Thrombocyte (Platelet) Count.

Result:

Date	**Species**	**Thrombocyte Count**

UNIT-1

Practical No.13 **Date:**

DETERMINATION OF BLOOD GROUP

Blood group typing in human beings:

There are large numbers of blood group systems, based on related antigenic groups. The various blood group systems are ABO, Rh (D), MNSSU, P, Kell, Duffy, Kidd, Lutheran, Lewis, Li and Xg. Out of all these blood group systems, the two most important are ABO and Rh systems of blood groups.

Objective:

Determination of blood group of the given sample

Principle:

Procedure used is based on the principle of agglutination of R.B.C. possessing antigens, in the presence of specific antibodies.

Materials:

1) Clean, dry glass slides or test-tubes of 10x75mm size.
2) Applicator sticks.
3) Fresh blood sample.
4) Anti-A, Anti-B and Anti-D sera. (Rh).
5) Centrifuge machine.
6) Normal saline solution.
7) Pasteur pipette.

Procedure:

Slide method:

- Place a drop of Anti-A blood grouping serum on one half of a glass slide. On the other half of slide, place a drop of Anti-B blood grouping serum.
- On the other slide place a drop of Anti-D serum.
- A small drop of blood is placed alongside the sera on the two slides; which are then gently mixed.
- Mix well the blood sample and serum mixture with applicator stick. Use separate applicator sticks for each sample.
- Incubate the mixture for about two minutes.
- The slides are then rocked and examined. Agglutination is “positive” when the mixture shows a fine or coarse granular appearance which can be easily seen by naked eye. In case of doubt the preparation should be examined under microscope.

Agglutination of the red blood cells in presence of antiserum is a positive test and indicates the presence of corresponding antigen. If there is no agglutination of R.B.C. it is negative test and indicates the absence of corresponding antigen.

Tube Method:

- ❖ Prepare 5% suspension of red cell in isotonic saline.
- ❖ Take 1 drop of anti-A blood grouping serum in one test tube test.
- ❖ To a second tube, take 1 drop of Anti-B blood grouping serum.
- ❖ Using proper pipette add 1 drop of 5% cell suspension to each of the two tubes.
- ❖ Mix well of centrifuge for one minute at 1000 RPM.
- ❖ Then examine for agglutination.

Interpretation:

Sample no.	**Reactions with**			**Blood group**
	Anti –A serum	**Anti-B serum**	**Rh**	
1	Negative	Negative	+	"O Rh^+ "
2	Positive	Negative	-	"A Rh^-"
3	Negative	Positive	+	"B Rh^+ "
4	Positive	Positive	-	"AB Rh^- "

If reaction with Anti-D is positive i.e. Rh Positive.
If reaction with Anti-D is negative i.e. Rh Negative.

"A" antigens are further classified as "A_1" and "A_2" sub groups. The differentiation of "A_1" and "A_2" cells can be done by using corresponding antisera.

Rhesus system of blood groups:

- ❖ Next to "ABO" system is the Rhesus system of blood groups. Because the antigens of this system were first detected in Rhesus monkeys, this system was named as so.
- ❖ There are five Rh antigens viz- C, c, D, E, e. Groups containing dominant antigens i.e. C,D and E will be Rh positive. Groups "C" and "E" seldom remain without "D". Hence all Rh positive cases contain Antigen "D". Hence, an Anti-D serum is sufficient to know whether the sample is Rh positive or not. The Rh

negative cases contain recessive agglutinogensViz:c and e. All persons can be classified in to three phenotypic types on the basis of the “D” antigen. Rh positive, Rh negative and weak Rh positive. Weak Rh positive reaction is due to the presence of a “D” variant.

Blood group systems in domestic animals

Species	Blood group system	Technique used for blood typing
Cattle	12	Hemolytic
Sheep	8	Hemolytic and agglutination
Horse	8	Hemolytic and agglutination
Pig	15	Hemolytic and agglutination
Dog	11	Hemolytic and agglutination
Cat	2	Hemolytic and agglutination
Chicken	12	Hemolytic and agglutination
Human	14	Hemolytic and agglutination

Precautious:

1. Excess amount of blood should be avoided for clear clump formation.
2. Antibody droppers should not be touched with blood during addition of antiserum.
3. Mixing of blood and antiserum must always be done with separate stirrer.
4. Do not allow blood to dry up during reaction.

Significance of blood grouping:

1. In the blood transfusion.
2. In the problems of identity and parentage.
3. In the study of genetics.
4. In the association and proneness to disease.

Observation:

UNIT-1

Practical No.14 **Date:**

ERYTHROCYTE OSMOTIC FRAGILITY TEST

Objective:

Determination of osmotic fragility of erythrocytes

Principle:

When the red cells are suspended in the buffered hypotonic saline, they swell up and ultimately rupture. This is known as haemolysis. The intensity of haemolysis is proportionate to the degree of hypo tonicity of the solution, which could be measured calorimetrically.

Materials required:

1) 10% buffered Sodium chloride stock solution.

NaCl - 9.0gm

NaH_2PO_4 - 1.366gm

Na_2HPO_4 - 1.870gm

Distilled water - 1000ml

Prepare working solution (1% buffered NaCl solution) by mixing 10ml of stock solution with 90ml of distilled water.

2) Pipettes. 3) Test tubes (10ml capacity). 4) Centrifuge machine.

5) Colorimeter. 6) Distilled water. 7) Anticoagulated fresh blood sample.

Procedure:

- ❖ Number the 10ml capacity test tubes serially from 1 to 16.
- ❖ Dilute 1.0% buffered NaCl solution in the range of 0.1 to 0.85% as follows:

Tube No.	1% NaCl (ml)	Dist. Water (ml)	NaCl %	Optical density
01.	4.25	0.75	0.85	
02.	4.00	1.00	0.84	
03.	3.75	1.25	0.75	
04.	3.50	1.50	0.70	

05.	3.25	1.75	0.65	
06.	3.00	2.00	0.60	
07.	2.75	2.25	0.55	
08.	2.50	2.50	0.50	
09.	2.25	2.75	0.45	
10.	2.00	3.00	0.40	
11.	1.75	3.25	0.35	
12.	1.50	3.50	0.30	
13.	1.25	3.75	0.25	
14.	1.00	4.00	0.20	
15.	0.50	4.50	0.10	
16.	0.00	5.00	0.00	

- With the help of hemoglobin pipette add 0.02ml (20ul) of thoroughly mixed blood to each tube.
- Mix by inverting the tube gently. Ensure proper mixing of blood with the saline.
- Allow the tubes to stand at room temperature for 30 minutes.
- Centrifuge all tubes at 2000rpm for 10 minutes.
- Collect the supernatant fluid and read the colour intensity in the form of optical density at 540nm by using either spectrophotometer or colorimeter. Use distilled water as blank.
- The haemolysis in tube 16 is regarded as being 100%.
- Convert the optical density to percent haemolysis as follows:

$$\text{Percent haemolysis} = \frac{\text{O.D. of unknown X 100}}{\text{O.D. of tube 16}}$$

- The haemolysis is expressed by two readings. The point of minimum resistance is that concentration of NaCl where slight haemolysis is detectable. The point of maximum resistance is that concentration of NaCl at which haemolysis is completed.

UNIT-1

Practical No.15 **Date:**

MEASUREMENT OF BLOOD PRESSURE

Objective: Determination of blood pressure by using Sphygmomanometer.

Principle: Blood pressure is the lateral pressure exerted by blood on the vessel walls while flowing through it.

Procedure:

Clinically the blood pressure is measured by indirect method using a Sphygmomanometer. Three indirect methods are used for recording blood pressure:

- **Oscillatory**
- **Palpatory**
- **Auscultatory**.

There are two types of sphygmomanometers viz- Mercurial and Aneroid. Both the types essentially consists of the following parts viz-

1. **Inflatable cuff**: an armlet made up of a rubber bag encased in non distensible silk fabric.
2. **Pressure bulb:** made up of rubber having screw valve for releasing the pressure.
3. **Manometer:** may be either mercurial or aneroid type. Measures the pressure developed inside the cuff by the bulb.

1) Oscillatory method:

- Wrap the inflatable cuff over brachial artery and keep the instrument at the level of heart.
- By using pressure bulb, increase the pressure in cuff. When the cuff pressure is increased and raised above the systolic pressure, the oscillations disappear. But on releasing the pressure gradually, the oscillations become larger and prominent. The pressure at which larger oscillations are produced by the pulsations is considered as systolic pressure. But on release of pressure, the oscillations become smaller and disappear. The pressure at which oscillations become smaller and disappears is taken as diastolic pressure.

2) Palpatory method:

Feel the radial artery with the fingers. Inflate the cuff by pumping air with pressure bulb until the radial pulse disappears. Raise the pressure 10-20 mm above this pressure. Release the pressure slowly and note the pressure at which pulse reappears. This is considered as systolic pressure. In this method, diastolic pressure cannot be determined.

3) Auscultatory method:

Principle:

Streamline flow is silent. But the increased velocity of the flow through the constricted artery results in turbulence and production of sound. The characteristic sounds generated by the blood flow at the compressed arterial location are called “Korotkow sounds”.

Procedure:

- Place the stethoscope (chest piece) in the antecubital fossa (human) over the brachial artery about half inch below the lower border of the sleeve. No sound will be heard over the normal uncompressed artery.
- Now inflate the cuff. On inflating the cuff, the sounds of vibrating artery will be heard, gradually growing fainter as the external pressure is increased.
- Observe the point of disappearance of sound and raise the pressure (about 10-20mm) above this point.
- The brachial artery is occluded by the pressure and no sound is heard with stethoscope. Lower the pressure slowly (2-3mm per heart beat) until a clear faint, tapping sound is heard.
- The manometric pressure at the reappearance of first tapping sound coincides with the systolic pressure.
- When the pressure is further released, normal streamline flow sets in and the sound is no longer heard. At this point the manometric pressure coincides with the diastolic blood pressure.
- As the pressure in the cuff is progressively lowered the sound undergoes a series of changes in quality and intensity. The sequential changes in the sound are

known as “Korotokow sounds”. Four phases of sound (sounds of Korotkow) may be heard in succession in the normal individual as the pressure is gradually recorded from about 120-80mm of mercury or less.

Sounds of “Korotokow”

Phase 1 (120-110 mm):

Sudden appearance of clear, faint, tapping sound grows louder as the pressure decreases gradually from 120 mm of mercury.

Phase 11 (110-95 mm):

During this phase tapping sounds are replaced by a murmur as the pressure decreases gradually from 110 mm of mercury.

Phase 111 (95- 80):

The murmur is replaced by clear loud gong as the pressure decreases gradually from 95 mm of mercury.

Phase 1V (80 mm):

The loud sound suddenly becomes dull, muffled and rapidly begins to fade. This point indicates diastolic blood pressure.

The beginning of the first phase is taken as systolic pressure. The pressure at the time of complete disappearance of the fourth sound i.e. the end of phase IV is taken as diastolic pressure. Another view regarding measurement of diastolic blood pressure is that the point where the third phase (clear, loud gong sound) is transformed in to the forth phase (dull, muffled sound) i.e. the beginning of forth phase is taken as the index of diastolic B.P.

If this change cannot be appreciated then add 5mm to the reading of complete disappearance of sound and take this as diastolic B.P.

Precautions:

- ❖ Keep the instrument always at the level of the heart.
- ❖ Avoid improper positioning of stethoscope head on limb. Keep it 2 cm away from the lower edge of the cuff.
- ❖ Do not employ high cuff pressure for a prolonged period, which increases the B.P. of an animal due to reflex mechanism.
- ❖ Record B.P. in normal resting animal.

- ❖ Lateral pressure is that pressure when force is exerted at right angles to the direction of flow at any point within a tube filled with a circulating fluid.
- ❖ Systolic pressure is the highest pressure during systole.
- ❖ Diastolic pressure is the minimum pressure during diastole.
- ❖ Pulse pressure is the pressure between systolic and diastolic pressure.
- ❖ Mean pressure is the arithmetic mean of the diastolic and systolic pressure.
- ❖ Basal B.P. is the B.P. of an individual with least possible amount of stress/strain.

Method of determination of arterial blood pressure:

There are two methods: 1) Direct 2) Indirect method.

1) **Direct method**: A canula is inserted inside the artery and is connected to U shaped mercurial manometer that shows the actual pressure in mm of mercury. For recording B.P. a floating stylus with a writing pointer that marks on the smoker paper may be used.

Horse & Cattle: Brachial artery.

Dog: Femoral artery.

OBSERVATION & RESULT:

UNIT-1

Practical No.16 **Date:**

RECORDING OF ELECTROCARDIOGRAM

Electrocardiogram is a graphic record of voltage fluctuations produced by myocardium during cardiac cycle. It represents electrical activity of heart and it precedes the mechanical activity.

Heart is an electrical field in which currents flow in repetitive patterns with each cardiac cycle. The arms and legs are linear extensions of this electrical field. Therefore electrical activity of heart can be detected at extremities by placing electrodes of opposite polarity on the skin at opposite poles of the heart. Two electrodes of opposite polarity or one electrode and a reference point compose lead. The leads are attached to an amplifier within an oscilloscope.

Modern E.C.G. records a calibrated standard 12 lead electrocardiogram. The 12 leads are: 3 bipolar lead viz: I, II, III; 3 augmented unipolar limb leads viz: $_aV_{R,}$ $_aV_{L,}$ $_aV_{F,}$ and 6 unipolar chest leads viz: V_1, V_2, V3, V_4, V5 and V_6. Bipolar leads and unipolar limb leads are frontal plane leads, which give information about current flow in frontal plane i.e., right, left, inferior and superior but not about anterior or posterior forces, which can be recorded by horizontal plane leads i.e., by unipolar chest leads. Bipolar lead is composed of two electrodes one positive and other negative placed at an equidistant from heart with each electrode contributing equally to the tracing. Unipolar lead comprises one positive electrode and a reference point which is neutral and is achieved by connecting 3 electrodes on limbs.

Bipolar leads:

Lead I: The negative electrode is on the right arm and the positive electrode on the left arm. Thus, the axis of lead I run from right shoulder to left shoulder.

Lead II: The negative electrode is on the right arm and the positive electrode on the left leg. Thus, the axis of lead II runs from right shoulder to left leg.

Lead III: The negative electrode is on the left arm and the positive electrode on the left leg. Thus, the axis of lead III runs from left shoulder to left leg.

Unipolar limb leads:

There are 3 unipolar limb leads viz-$_aV_R$, $_aV_L$ and $_aV_F$. Eliminating a negative electrode results in 50% augmentation of the amplitude that is recorded. Hence these unipolar limb leads are called augmented unipolar limb leads. The letter "a" stands for augmented. "V" indicates a unipolar lead. The letters "R, L, F" indicate where the positive electrode is placed i.e., the right arm, left arm or left leg.

Precordial or unipolar chest leads:

The precordial leads are unipolar horizontal plane leads and reflect anterior and posterior forces. There are 6 precordial leads viz: V_1, V_2, V_3, V_4, V_5 and V_6. Each lead provides a different view of the heart's electrical activity. The placement of precordial chest leads is as follows:

V_1– Right 4th intercostal space one inch away from right sternal border.

V_2 –Left 4th intercostal space one inch away from left sternal border.

V_3 – At the mid point between V_2 and V_4.

V_4 – Left 5thintercostals space at mid clavicular line.

V_5 –Left anterior axillary line in the level of V_4.

V_6– Left mid axillary line in the level of V_4 and V_5.

If an imaginary line is drawn between the two electrodes of opposite polarity or between an electrode and the reference point, the line would be axis of the lead. The currents generated by the heart cause certain deflections on the ECG according to how they relate to the axis of the lead. When the main current flow of the heart (electrical axis of heart) is parallel to the axis of lead, the resulting ECG complex is either the most positive or the most negative deflection of all, depending upon whether it flows towards the positive or negative electrode. When the main current flow of heart is perpendicular to the axis of the lead, it is neither positive nor negative i.e., an isoelectric (equiphasic) deflection will be written by the ECG. (An equiphasic deflection is one i.e., equally positive and equally negative).

Einthoven's triangle is formed by the axes of the three bipolar limb leads I,II and III. Einthoven's law states that the complex in lead II is equal to the sum of the complexes in leads I and III.

$$I + III = II$$

$$I + II + III = 0.$$

Conduction system and the Electrocardiogram :

The genesis of the Electrocardiogram involves number of factors viz:--

i) Initiation of impulse formation in the primary pacemaker (S.A. node).

ii) Transmission of the impulse through the specialized conduction system of the heart.

iii) Activation or depolarization of the atrial and ventricular myocardium.

iv) Recovery or depolarization of atrium and ventricles.

The normal E.C.G. is composed of a “P” wave, a “QRS” complex and “T” wave. The “QRS” complex is often three separate waves, the “Q” wave, the “R” wave and the “S” wave.

“P” wave represents atrial depolarization. Depolarization waves spread from S.A. node through the right atrium towards the left atrium and A.V. node in a radial direction like a ripple in a pond. The result is a positive wave (P wave) because the depolarization or activation force is directed towards the positive electrode.

“QRS” complex represents ventricular depolarization. The “Q” wave, first phase of ventricular depolarization represents septal depolarization. The depolarization force transmitted by left bundle branch is directed towards right bundle branch i.e., the depolarization forces are directed from left to right side of the septum i.e., away from the positive electrode resulting in a negative deflection (Q wave). “R” wave, second phase of ventricular depolarization is a positive wave. The conduction system branches sub endocardially, with the apex and free walls of both ventricles simultaneously depolarized from the endocardium towards the epicardium. Because the mass of the left ventricle exceeds that of the right, leftward electrical forces predominate over those directed to the right. The spread of the impulse towards the positive electrode though the left ventricular muscle mass causes the upward deflection i.e. “R” wave. The third phase of ventricular depolarization produces the “S” wave. The base is the last part of the ventricles to be activated. Since the depolarization wave moves away from the positive electrode a negative deflection i.e. “S” wave is recorded. “T” wave represents ventricular repolarization. The greatest portion of ventricular muscle to repolarize first is that located over the

entire outer surface of the ventricles and especially near the apex of the heart. The endocardially areas on the other hand normally repolarise last. Because the outer and apical surfaces of the ventricles repolarise before the inner and basal surfaces, the positive end of the heart vector during repolarization is towards the apex of the heart. Thus, the predominant direction of the vector through heart during repolarization of the ventricles is from base to apex, which is also the predominant direction of the vector during depolarization. As a result the “T” wave in the normal bipolar limb leads is positive, which is also the polarity of most of the normal “QRS” complex.

Repolarization of atria:

The area in the atria that becomes depolarized first is the S.A. nodal region, the area that had originally become depolarized first. When the depolarization begins, the region around S.A. node becomes positive with respect to the rest of the atria. Therefore, the atrial depolarization vector is backward to the vector of depolarization causing negative deflection i.e. “Ta” wave. In normal E.C.G. “Ta” wave is not visible because atrial repolarization occurs almost simultaneously with ventricular depolarization. Therefore “Ta” wave is almost always totally obscured by the larger “QRS” complex.

The first region in the ventricle to get stimulated is the mid portion of interventricular septum on left side. From this side it passes through the septum to right side and then the impulse spreads to the apex on either side first to right apex and then to left apex. From the apex it spreads to the base of ventricles on either side. In this process the impulses also spread from endocardium to epicardium. The last regions to be excited are pulmonary conus, upper portion of interventricular septum and lastly posterobasal region of left ventricle. When ever the electrical potential of heart travels in the direction of positive electrode a positive deflection or wave will be recorded on the paper and when it moves away towards negative electrode a negative deflection or wave is obtained.

Normal electrocardiogram recorded from various leads

Record from Bipolar limb leads:

E.C.G. recorded simultaneously in three bipolar leads show similar features “P”, “R”, “T” waves are positive waves whereas “Q”, “S” waves are negative in all

three bipolar limb leads. In lead- I, “R” is mainly caused by right ventricle and “S” wave due to left ventricle. In lead- III, it is just the reverse i.e., “R” wave is due to left ventricle and “S” wave due to right ventricle.

Normal Electrocardiogram recorded from Precordial leads*:*

The different recordings recorded by leads V_1, V_2, V_3, V_4, V_5 and V_6 are shown in figure: 5. In leadsV_1 and V_2 the “QRS” recordings of normal heart are mainly negative because the chest electrode in these leads is nearer to the base of the heart, which is the direction of electro negativity during most of the ventricular depolarization phase. On the other hand, the “QRS’ complex in leads V_4,V_5 and V_6 is mainly positive because the chest electrode in these leads is nearer to the apex, which is the direction of electropositivity during most of the ventricular depolarization.

Normal Electrocardiogram recording from unipolar limb leads:

Normal recordings of the augmented unipolar limb leads are shown in Fig:5. These are all similar to the bipolar limb lead recordings except that recording from the $_aV_R$lead is inverted. The reason for this inversion is that the polarity of the electrocardiograph in this instance is connected backward to the major direction of current flow.

Significance of various leads:

1) **Standard bipolar limb leads:**
   - ❖ Valuable for diagnosis of arrythmia and also for preliminary study of functional abnormalities of heart.

2) Precordial leads (Unipolar chest leads): are important
   - ❖ for localization of the recent or old ventricular damage
   - ❖ Bundle branch block
   - ❖ Detection of ventricular hypertrophy.

3) **Augmented unipolar limb leads:** are valuable for
   - ❖ Determining the position of heart.
   - ❖ Confirming the significance of “Q” and “T” waves in the standard lead.
   - ❖ Confirming the evidence of ventricular damage or hypertrophy.
   - ❖ E.C.G. appearance under certain cardiac disorder:

1) “P” wave becomes larger in the condition **atrial hypertrophy.**

2) Inverted “P” wave indicates the atrial muscle depolarizes by the impose originating in A.V. node i.e. an ectopic pace maker.

3) “P” wave absent in **atrial fibrillation**.

4) **Bradycardia**: slow heart beat <60 beats/minute.

5) **Tachycardia:** fast heart beat >100 beats/minute.

6) **Sinoarrythmia**: the pacemaker is the S.A node and conduction is normal. However, the rhythm is irregular which alternately slows and then speeds up.

7) **Sinoatrial block (S.A. block):** Impulse from S.A node is blocked before it enters atrial muscle resulting in the sudden cessation of “P” waves. Ventricles pick up new rhythm by the impulse usually originating in A.V. node.

8) **Atrio-ventricular block**: Impulses pass from atria to ventricles through A.V. bundle (bundle of His). The different conditions that can either decrease the rate of conduction or can totally block the impulse through this bundle are:

- ❖ Extreme stimulation of heart by vagus.
- ❖ Ischemia of A.V. nodal fibers.
- ❖ Compression of the A.V. bundle by scar tissue.
- ❖ Inflammation of A.V. node or A.V. bundle

Incomplete heart block (first-degree block):

P-R interval is prolonged,increases from 0.16 to 0.38 seconds.

Second degree block (Partial A.V. block) (Wenckebach phenomenon):

In the second degree block “QRS” complex follows each second or third “P” wave (2:1 or 3:1 heart block). That is in this form of heart block there will be drop of ventricular beats.

Complete A.V. block (III degree block):

In this type the impulse conduction is completely interrupted due to damage of bundle of His. There are complete Atrio ventricular dissociations. Atrium beats at the rate of sinus rhythm i.e. 80-100 beats/minute while the rate of ventricular beat is <40 beats/minute. In E.C.G. P-P interval and R-R interval will be same but P-R interval will be variable because “P” waves completely dissociates from the “QRS” complex.

9) **Ventricular premature beats/Extra systole**:

Sometimes portion of myocardium becomes irritable and ectopic beat occurs before the expected next normal beat. This ectopic beat causes transient interruptions of the cardiac rhythm.

10**)** **Ventricular paroxysmal tachycardia**:

When there is considerable damage to myocardium, series of ventricular premature beats occur successively for several beats without any normal beat interspersed. This is the early stage of ventricular fibrillation.

11) **Ventricular fibrillation**:

When there is massive damage at multiple areas of the ventricles, the complexes are bizarre and individual components cannot be deciphered.

12) **Ischemic heart disease**:

When the circulation to heart muscle is interfered, in the ECG, there is inversion of “T” wave. The RS-T segments are sagged.

13) **Myocardial infarction**:

Area of muscle is necrosed due to permanent arrest of blood supply to that area. In this condition, E.C.G. shows:

- Deep “Q” wave.
- S-T segment elevation.
- “T” wave inversion.

14) **Premature atrial contraction:**

- “P” wave and “QRS” complex is normal.
- P-R interval and interval between preceding contraction and premature contraction shortens. The interval between premature contraction and next succeeding contraction is slightly prolonged which is called “compensatory pause”.

15) **Atrial flutter**:

The rate of atrial contraction (P wave) is approximately 300 times/ minute while the rate of ventricular contraction is 125 times/ minutes i.e. the ratio of “P” waves to “QS” complex is either 2:1 or 3:1.

16) **Atrial fibrillation**:

During atrial fibrillation, numerous small depolarization waves spread in all directions through atria. Because the waves are weak and because many of them are of opposite polarity at any given time, they usually almost completely neutralize ach other. Therefore in E.C.G. “P” waves are absent whereas “QRS” complex is completely normal.

17) **Ventricular hypertrophy**:

Axis is generally deviated towards the hypertrophied side. This is due to increase of muscle mass in the hypertrophied side. The depolarization wave takes much longer time to cover the hypertrophied side than the normal one. Consequently, the normal ventricle becomes depolarized i.e. becomes negative considerably in advance of hypertrophied ventricle, and this causes a strong vector from the normal side of the heart towards the hypertrophied side, which is still positively charged. Thus, the axis deviates towards the hypertrophied ventricle.

Left ventricular hypertrophy:

Occurs due to hypertension, which causes the left ventricle to hypertrophy in order to pump blood against the elevated systemic arterial pressure. Also occurs in conditions such as aortic- valvular stenosis, aortic valvular regurgitation.

Right Ventricular hypertrophy:

Occurs due to pulmonary stenosis, increased pulmonary vascular resistance.

18) **Bundle branch block**:

In bundle branch block the axis is deviated towards the side of the block. In normal condition the impulse is transmitted to the lateral walls of two ventricles through the two branches almost at the same instant and two ventricles depolarize almost at the same time. If right bundle branch is blocked, the left ventricle depolarize far more rapidly than the right ventricle, so that left ventricle remains electronegative while the right ventricle remains electropositive. Therefore a strong vector develops with it's negative end towards the left ventricle and positive end towards right ventricle i.e., right axis deviation occurs if there is right bundle branch block and left axis deviation occurs if there is left bundle branch block.

In the normal human heart the mean electrical axis (+50°) will be found lie between -30° to +120° (ref. Fig's 7, 8, 9 &10). In the normal canine heart the mean electrical axis will be found to lie between +40° and +100°. In the normal feline heart the mean electrical axis will be found to lie between 0° and+100°

When a vector is horizontal and directed toward the subject's left side, the vector is said to extend in the direction of zero degree. From this zero reference point, the scale of vector rotates clockwise; when the vector extend from above downward, it has a direction of +90°; when it extends from the subject's left to the right, it has a direction of +180° and when it extends upward, it has a direction of -90° or +270°.

Duration of electrocardiogram intervals in animals, Lead II

Animal	P	PR	QRS	QT
Horse	0.08 – 0.16	0.19-0.39(0.28)	0.07-0.14(0.10)	0.34-0.57(0.45)
Cattle	----------	0.10-0.30(0.19)	0.06-0.12(0.09)	0.29-0.47(0.39)
Dog	----------	0.06-0.13(0.10)	0.04-0.07(0.05)	0.14-0.20(0.18)

❖ Range in seconds, average shown in parentheses.

OBSERVATION & RESULT:

Appendix :1. Normal Haematological Values in Domestic Animals andPoultry.

Parameters	Cattle	Buffalo	Sheep	Goat	Horse	Dog	Cat	Pig	Camel	Fowl
Hb (gm/dl)	8.0-14.0	-	8.0-16.0	8.0-14.0	11.0-18.0	12.0-18.0	8.0-15.0	10.0-16.0	9.0-15.0	-
PCV (%)	25-40	-	25-45	20-39	32-52	37-55	25-45	30-50	25-35	-
ESR (mm/hr)	0-1	-	0-1	0-1	15-40/20min.	6-11	55	1-15	2.5/3hrs	1-3
MCV (fl)	40-60		25-50	15-25	35-60	60-78	40-55	50-70	25-50	-
MCH (pg)	15-20	-	10-14	8.0-10	13-19	20-24	13-17	16-20	13-18	-
MCHC (g/dl)	32-39	-	27-36	32-34	33-35	30-35	32-34	28-35	40-45	-
TEC ($\times 10^6$/µl)	5.4-9.0	5.4-7.4	8.5-13.5	8.0-17.0	6.5-13.0	6.4-8.0	6.2-10.0	5.0-9.0	7.0-13.0	2.8-4.5
TLC ($\times 10^3$/µl)	4.5-13.0	8.0-10.0	4.0-12.0	5.0-13.0	5.0-11.0	6.4-15.0	5.5-19.0	8.0-20.0	10.0-19.0	20.0-40.0
Neutrophils (%)	25-30	-	25-30	35-40	50-60	65-70	55-60	30-35	65-75	25-30
Eosinophils (%)	2.0-5.0	-	2.0-5.0	2.0-5.0	2.0-5.0	2.0-5.0	2.0-5.0	2.0-5.0	3.0-6.0	3.0-8.0
Basophils (%)	<1	-	<1	<1	<1	<1	<1	<1	1.0-2.0	1.0-4.0

Lymphocyt(%)	60-65	-	60-65	50-55	30-40	20-25	30-35	55-60	28-38	55-60
Monocytes (%)	5.0	-	5.0	5.0	5.0-6.0	5.0	5.0	5.0-6.0	4.0-5.0	10.0
Clotting time (min.)	3-15 (5)	-	1-6 (2.5)	2.5-11.5 (2.5)	3-15 (5)	3-4 (2.5)	-	2.5-4.0 (3.5)	5-7 (5.5)	4-5 (4.5)

Appendix :2. Normal Physiological Parameters in Domestic Animal and Poultry.

Species	**Arterial blood pressure(mm/Hg)**	**Respiratin rate /min.**	**Heart rate/min.**	**Temperature**	
				^{0}C	**^{0}F**
Cattle	140 : 95	30 – 32	40 – 60	37.5 - 39.0	99.5 - 103.0
Buffalo		30 – 40	50 – 70	37.5 - 39.0	99.5 - 102.2
Sheep	120 : 70	12 – 15	70 – 80	38.5 - 40.0	101.3 - 104.0
Goat		12 – 15	70 – 80	38.5 - 40.5	101.3 - 104.9
Horse	110 : 60	09 – 10	28 – 40	37.5 - 39.0	99.5 - 101.3
Dog	130 : 85	14 – 16	60 – 80	37.5 - 39.0	99.5 - 102.2
Cat		20 – 30	110 – 130	38.0 - 39.5	100.4 - 103.1
Pig	150 : 100	14 – 16	60 – 90	38.0 - 40.0	100.4 - 104.0
Camel		05 – 12	30 – 50	35.0 - 38.6	95.0 - 101.5
Fowl (Chicken)	195 : 155	15 – 30	120 – 160	40.5 - 43.0	104.9 - 109.4

UNIT-2

Practical No.1 **Date:**

COUNTING OF RUMEN MOTILITY

Principle:

The orderly and synchronized movements of the reticulum and rumen help in mixing the fresh ingesta with that already present in the stomach and propelling the food downwards and simultaneous uplifting of the gases in the dorsal sac and towards cardia for eructation.

Methods: The motility or contractions of the rumen can be identified and counted by

1. Visual inspection method
2. Palpation method

Materials required:

1. Animal
2. Service crate

Procedure:

1. Visual inspection method:-

1. Restrain the animal in a service crate.
2. Observe the left paralumbar fossa of the animal for movements.
3. The left paralumbar fossa rises and sinks down during rumen contraction.
4. The number of movements per 5 minutes gives the number of rumen contractions.

2. Palpation method:

1. Restrain the animal in a service crate.
2. Stand on the left side of the animal.
3. Press your fist in the left paralumbar fossa.
4. Feel the movements of the rumen in your fist.
5. Count the number of rumen movements per 5 minutes.

Observation/Result:

UNIT-2

Practical No.2 **Date:**

COLLECTION OF RUMEN LIQUOR

The liquor or fluid from the rumen is collected for the following purposes:

1. To diagnose the various digestive disorders in the ruminants

Examining the pH, the population of microbes (Bacteria, Protozoa and Fungi), the total volatile fatty acids, total ammonia nitrogen etc. of the rumen liquor can help in the diagnosis of various diseases.

2. As a treatment procedure:

In conditions like rumen acidosis removal of the contents of the rumen can save the animal.

3. For cud transplantation:

The population of microbes in the rumen liquor will be very low or even nil in sick animals. The collection of rumen contents from healthy animals and transferring it to the sick animals will reestablish the normal microbial population in the rumen of the sick animals. This procedure is called as cud transplantation.

Methods of collection:

The most commonly used methods for collection of rumen liquor are

1. Stomach tube method.
2. Rumen Fistula method.
3. Hypodermic needle method

1. Stomach tube method:

Materials required:

A specially designed suction pump, a three meter long nylon stomach tube, an air tight sampling beaker, liquid paraffin.

Procedure:

1. Apply liquid paraffin over the stomach tube for lubrication.
2. Restrain the animal.
3. Open the mouth of the animal by pulling out the tongue to one side.
4. Hold the head of the animal high and introduce the tube (intubation).
5. Pass the tube over the tongue and past the epiglottis into the cranial part of the esophagus.
6. Take care to avoid passing the tube into the trachea and causing any injury.
7. Keep on pushing the tube gently until it reaches the rumen.
8. When the stomach tube touches the rumen contents connect the tube with the suction pump.
9. Operate the suction pump and collect the required quantity of rumen liquor into an air tight sampling beaker.

Advantages:

1. No surgical manipulations are required.
2. This method is best suited for clinical studies.

Disadvantages:

1. The collection procedure is difficult.
2. Samples representing the different regions of the rumen cannot be collected because only the anterior portions of the rumen will be accessible to the stomach tube.
3. The rumen liquor collected will be mixed with large amounts of saliva because the insertion of the stomach tube will stimulate the secretion of saliva.
4. Samples cannot be collected frequently using this method because repeated insertions of the stomach tube can injure the mucous membrane of the esophagus.
5. This method is not suited for experimental studies.

Precautions:

1. Apply liquid paraffin over the stomach tube before insertion.
2. Stomach tube should not be directed towards trachea during insertion.

2. Rumen fistula method:

The term fistula refers to an artificial opening. The animal is operated on the left flank and the fistula is fixed. The fistula may be a plastic or metal device and it consists of a hollow tube, inner and outer plates and a cap.

Materials required:

A fistulated animal, a hard plastic tube attached with rubber bulb or plastic tube attached with suction pump and container.

Procedure:

1. Restrain the fistulated animal in a service crate properly.
2. Open the cap of the fistula.
3. Insert the plastic tube into the rumen through the fistula.
4. Attach the tube with suction pump and collect the desired amount of fluid.
5. A plastic tube attached with rubber bulb can be used to collect small amounts of rumen liquor.

Advantages:

1. The collection procedure is very simple.
2. Samples representing the different regions of the rumen can be collected easily.
3. The rumen liquor collected is not mixed with saliva.
4. Samples can be collected frequently; round the clock without any problem to the animal.
5. This method is best suited for experimental studies.

Disadvantages:

1. Surgical operation is required to fix the fistula.
2. Post-operative care is needed to prevent the infection (Maggot formation).
3. The economic value of the animal is reduced.
4. This method is not suited for clinical studies.

5. The fistula often slips out or falls into the rumen due to changes in the size of the hole.

Precautions:

1. Apply antiseptic cream for the healing of wound.
2. Apply turpentine oil on the wound during maggot formation.

3. Hypodermic needle method

This method is adopted to collect the rumen liquirfom young ruminants under emergency. About 6 inch long with wide bore hypodermic needle attached with syringe is required for the collection of fluid.

Apparatus:

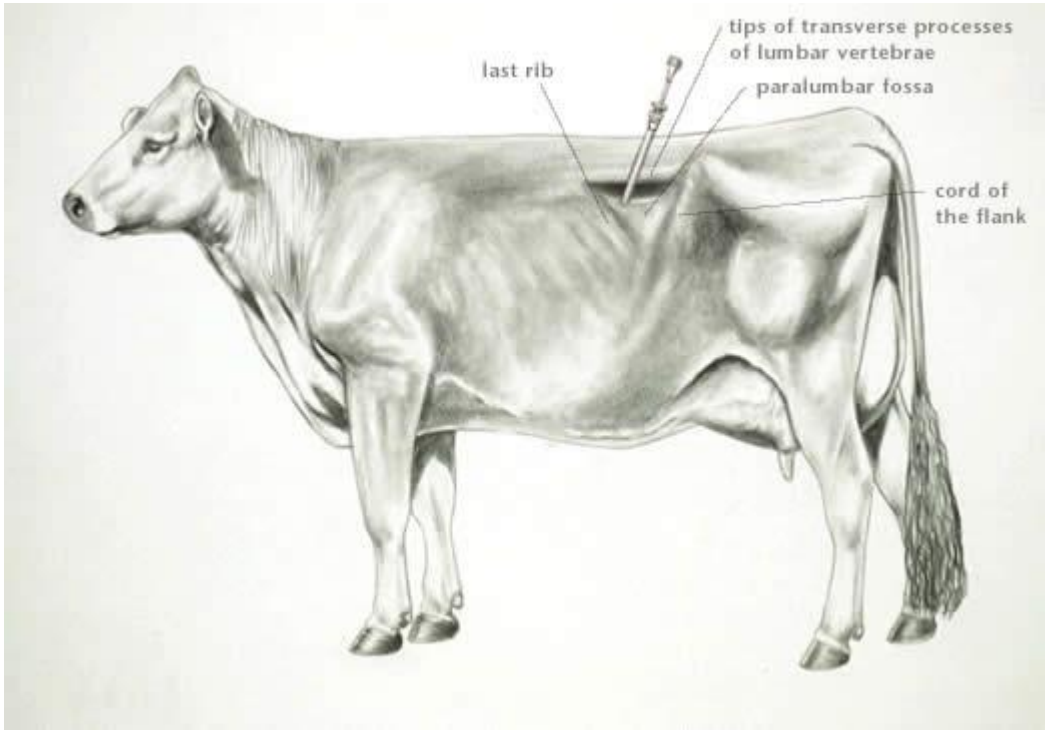
1. hypodermic needle
2. Syringe
3. Cotton and spirit
4. Container

Procedure:

1. Secure the animal properly and apply antiseptic on the left flank in the lower region of triangular area.
2. Insert the needle with single thrust in the rumen through the lower region of the triangular area.
3. Draw desire amount of rumen liquor in the syringe by pulling the plunger and transfer in to the collecting vial.
4. Retract the needle from the rumen and apply the antiseptic cream to prevent infection

Advantages:

1. It is a simple and easy way of obtaining the rumen liquor under emergency.
2. There is no need of surgical operation



Hypodermic needle method

Rumen liquor collected by rumen fistula

Disadvantages:

1. Representative sample of rumen liquor can not be collected by this procedure
2. repeated sampling can not be obtained, otherwise it will create injury to the rumen wall and peritoneum
3. It is limited to the young ruminants only.

Preparation of strained rumen liquor

1. Filter thecollected rumen liquorthrough two layers of muslin cloth.
2. The filtrate obtained is called strained rumen liquor (SRL).
3. This strained rumen liquor (SRL) is used for enumeration of rumen bacteria and protozoa, estimation of ammonia nitrogen, and determination of total volatile fatty acids (TVFA) content in rumen liquor.

Observations/Result:

UNIT-2

Practical No.3 **Date:**

ENUMERATION OF RUMEN BACTERIA IN RUMEN LIQUOR

Rumen bacteria play an important role in the digestion of food in ruminants. They can live at PH 5 to 7, in the absence of Oxygen, at the temperature 39-40^{0}c in presence of moderate concentration of fermentation product. Normal concentration of rumen bacteria varied from x10^9 to x10^{10} per ml of rumen liquor.

Objectives:

1. To know the normal population of rumen bacteria.
2. To study the postprandial changes in the count of rumen bacteria.
3. To study the changes in the population of rumen microflora under different dietary regimens.
4. To study the effect of drugs on the number of rumen bacteria.
5. To study the diurnal variation 9in the population of rumen microflora.
   Different methods have been adopted for total counting of rumen bacteria.

1. PetroffHausser Counting Chamber Method

Apparatus/Requirements:

A. Petroff-Hausser counting chamber, microscope, test tubes, test tube rack, pipette, RBC diluting pipette, rumen liquor, crystal violet stain.
B. Crystal violet stain is prepared by mixing 10 ml of saturated solution of crystal violet in alcohol with 100 ml of distilled water and filter through filter paper before use.

Procedure:

1. Collect rumen liquor through rumen fistula and filter through two layers of muslin cloth.
2. Draw rumen liquor sample upto mark 0.5 in RBC pipette and then dilute with crystal violet stain upto mark 101 to give a 1:200 dilution.
3. Mix the sample with stain thoroughly by rotating the pipette between the palms.
4. Discard few drops of sample before charging the counting chamber.

5. Charge the counting chamber by placing small drop of sample into it and allow the bacteria to settle for 15 minutes.
6. Counting is made in 25 squares in high power of microscope.
7. Bacteria appear as cocci or rod shape.

Bacterial count per ml can be calculated with following formula.

$$\text{Bacteria/ml} = \frac{\text{Total bacteria in N number of squares x 200 x 20,000,000}}{\text{N number of small squares counted}}$$

2. Nigrosine Method-1

Materials Required:

Strained rumen liquor (SRL), Hemocytometer slide with Neubaur counting chamber, Microscope, Gentian violet crystals, and Formaldehyde.

Procedure:

1. Take the strained rumen liquor (SRL) in a beaker.
2. To the SRL add a few drops of formaldehyde (to kill the microbes).
3. Then, add a few crystals of gentian violet (to stain the microorganisms).
4. Fill the red cell diluting pipette with the above SRL.
5. Charge the Neubaur counting chamber with the above fluid in the red cell diluting pipette.
6. Leave the chamber undisturbed for a few minutes to allow the dead microbes to settle.
7. Focus the RBC chamber under high power and count the number of microorganisms in the five medium squares as you count for RBC counting.
8. The bacteria will be smaller and counting them will be easy.
9. The protozoa are larger and counting them will be difficult.
10. Count the bacteria.

Calculation:

The volume of 5 Medium squares = 1/50 cu mm.

No of bacteria in 1 ml

of rumen liquor = N x 50,000

Where, N refers to the number of bacteria in 5 Medium squares.

Observations/Result:

UNIT-2

Practical No.4 **Date:**

ENUMERATION OF PROTOZOA IN RUMEN LIQUOR

Rumen contains ciliate protozoa. There are two groups of protozoa (i) Holotrichs (ii) Entodiniomorphs. Holotrinchs are characterized by presence of cilia all over the body. Cilia are singly inserted. Endodiniomorphs are characterized by lacking of cilia all over the body. Cilia are fused and restricted to mouth. Population of protozoa varied with types of ration. Holotrichs predominate on green fodder while Entodiniomorphs on concentrate ration. Normal population of rumen protozoa varied from 10^5 x 10^6 per ml of rumen liquor.

Objectives:

- To know the normal population of ciliate protozoa.
- To study the changes in the number of protozoa under different dietary feeding.
- To study the changes in the total counts of protozoa under pathological conditions.
- To study the effect of drugs on the protozoal population.
- To study the diurnal variation in the protozoal count.

Apparatus/Requirement:

A. Glass slide, cover slip, microscope, rumen liquor, test-tube, test tube rack, pipette, muslin cloth, funnel.
B. Lugol's Iodine solution is prepared by dissolving 5 gm of Iodine and 10 gm potassium iodide in 60 ml of distilled water, add 10 ml of formalin and 30 ml of glycerol to make the volume 100 ml.

Procedure:

1. Collect rumen liquor and filter through two layers of muslin cloth.
2. After shaking strain liquor, place 1 ml of sample through a wide bore pipette into a test tube.
3. Add 9 ml of Lugol's iodine solution.
4. Mix it gently and 0.1 ml of sample is transferred swiftly to a dry clean slide and spread under a glass cover of known area (24 x 60 mm).

5. Counting of protozoa is done under low power of microscope in a zigzag manner.
6. Thirty fields are counted per slide both for ease and accuracy and average count per field calculated.
7. Total protozoal count per ml can be calculated by following formula.

Total protozoa/ml Rumen liquor	=	Average Protozoa Counted Per field	x	No. of microscopic field (1000)	x Dilution factor (100)

Draw different types of protozoa:

Observation/Result:

UNIT-2

Practical No.5 **Date:**

DETERMINATION OF AMMONIA NITROGEN IN RUMEN LIQUOR

The level of rumen ammonia is critical for efficient microbial fermentation of feed. It determines the rate and efficiency of digestion of fibrous feeds. Recent reports suggest that rumen protozoa populations are also reduced when rumen ammonia levels are high. Rumen ammonia concentration can be used to diagnose a deficiency or excess of fermentable nitrogen in a diet.

This will indicate when urea supplements are required.

Principle:

When the strained rumens liquor (SRL) and the alkali (50% potassium carbonate) in the outer compartment of Conway diffusion cell mix, ammonia is released. The liberated ammonia diffuses and gets absorbed in the boric acid present in the inner compartment of Conway diffusion cell. This involves 3 steps.

1) Liberation of ammonia.
2) Diffusion of ammonia.
3) Absorption of ammonia.

Materials required:

Strained rumen liquor (SRL), Conway Diffusion Cell, Pipette, Burette, Incubator.

Reagents required:

1) 50% solution of potassium carbonate solution.
2) 2% boric acid solution containing indicator.
3) 0.01N Sulphuric acid.

Procedure:

1. Open the lid of the Conway diffusion cell, and observe the two compartments in it.
2. Pipette out 1 ml of 2% boric acid solution and transfer it into the inner compartment of the Conway diffusion cell.

3. Pipette out 1 ml of strained rumen liquor (SRL) and transfer it into one side of the inner compartment of the cell.
4. Pipette out 1 ml of 50% potassium carbonate and transfer it into the inner compartment on the side opposite to that of the rumen liquor.
5. Close the lid of the Conway diffusion cell.
6. Rotate the cell 4 – 5 times so that the rumen liquor and the potassium carbonate solutions in the outer chamber mix together.
7. Incubate the cell at 39 – 40°c for two hours in a incubator.
8. Remove the cell from the incubator and titrate the contents in the inner compartment against N/10 sulphuric acid.
9. The end point is the appearance of pink colour.
10. Note the volume of N/10 sulphuric acid consumed.

Normal levels of rumen ammonia nitrogen:

As a rule of thumb, rumen ammonia nitrogen should be at least 15-20 mg/100ml rumen liquor.

Calculation:

Ammonia –N (mg/100ml) = Number of ml of acid used x 0.14 x 100

Observations/Result:

UNIT-2

Practical No.6 **Date:**

DETERMINATION OF TOTAL VOLATILE FATTY ACIDS IN RUMEN LIQUOR

Volatile fatty acids (VFA s) are the end products of carbohydrate fermentation and amino acid catabolism in the rumen. They are the main source of energy for the ruminants. Principal volatile fatty acids present in the rumen liquor are acetic acid, prop ionic acid and butyric acid. They occur in a proportion of 68-70%, 16 to 18% and 12-14% respectively. Total concentration of volatile fatty acids varies from 80 to 120 mEq/L.

Acetic acid provides energy and for milk fat in lactating animals, propionic acid maintains blood glucose level. Butyric acid is metabolized in the rumen epithelium and form ketone bodies.

Objectives:

1. To know the normal concentration of total volatile fatty acids in the rumen liquor.
2. To study the changes in the status of TVFA under different dietary regimes and pathological conditions.
3. To study the effect of drugs on the concentration of TVFA.
4. To study the diurnal variations in the level of TVFA.

Materials required:

Strained rumen liquor (SRL), Markham's distillation apparatus, Pipette, Burette, Hot plate, Burette, Pipette, Rumen liquor, funnel, test tube, test tube rack, muslin cloth, phenolphthalein.

Reagents required:

5% oxalic acid solution,
10% potassium oxalate solution
0.01 N sodium hydroxide solutions.

Procedure:

1. Pipette out 1 ml of strained rumen liquor.
2. Transfer it into the cup of distillation apparatus.
3. Add 0.5 ml of 5% oxalic acid and 0.5 ml of 10% potassium oxalate solution into the sample.
4. Put the stopper and place small amount of water to make it air tight.
5. Close the outlet with the help of clamp.
6. Keep the boiling flask over the hot plate and steam the contents.
7. Collect about 75 to 100 ml distillate in the 100 ml of conical flask.
8. Remove the distillate and add few drops of phenolphthalein indicator.
9. Titrate the distillate against 0.01N sodium hydroxide.
10. End point is the appearance of pink color.
11. Note the volume of 0.01N sodium hydroxide consumed.
12. And calculate the total VFA concentration.

Calculation:

The total VFA concentration can be obtained by multiplying the volume of 0.01N sodium hydroxide consumed with 10.

TVFA in mEq/L = volume of 0.01NSodium hydroxide consumed X 10

Observation/Result:

UNIT-2

Practical No.7 **Date:**

DETERMINATION OF LUNG VOLUMES & CAPACITIES BY USING SPIROMETER

Objective:

To determine the lung volumes and capacities of the subject by using Spirometer.

Materials:

1) Volunteer.
2) Spirometer.
3) Spirit/ Alcohol.

Spirometer

This consists of a metal cylinder of adequate capacity (6 1trs) which is filled with water. A metal tube pierces through the bottom of the cylinder and rises above the level of the water. The other end of metallic tube is connected by rubber tubing with mouth piece into which the volunteer breathes. The Spirometer bell is another metallic cylinder of slightly smaller diameter. It is completely submerged in the water and its weight is balanced by a counter weight. The top of the Spirometer bell is connected by a cord running over a pulley to a writing lever, which records the level of the bell on slowly revolving drum. The volume of air can be read from the scale on pulley.

Procedure:

- Fill the water chamber with the water and lower the spirometer bell in it.
- Sterilise the mouth piece with Spirit or Alcohol.
- Apply nose clip. Ask the subject to make normal inspiration, and then an expiration of normal depth in to the chamber. Repeat three or four times and get an average for the tidal air.
- Make a normal inspiration, and then the greatest possible expiration in to the chamber and get an average for the supplemental air plus the tidal.
- Make the deepest possible inspiration, and then the greatest possible expiration kin to the chamber and get an average for the vital capacity (supplemental air plus the tidal air plus complemental air).

Pulmonary volumes:

1) **Tidal volume:** is the volume of air that is taken in or given out during quiet breathing.

2) **Inspiratory reserve volume (IRV)/Complementalair:**is the volumeof air that can be taken in by forced inspiration over and above the tidal volume.

3) **Inspiratory capacity**:is the tidal volume and the volume of air taken during maximum inspiratory effort.

4) **Expiratory reserve volume (ERV)/Supplemental air:**is the volume of air that can be breathed out by forced expiration after normal expiration.

5) **Vital capacity (VC):** is the volume of air that can be breathed out by forced expiration after taking forced inspiration.

VC = Supplemental air + Tidal air + Complemental air.

6) **Residual volume (RV):** is the volume of air which remains in the lungs after maximal expiration. It can only be expelled from the lungs by opening the chest wall and allowing the lungs to collapse.

7) **Functional Residual capacity (FRC):**is the volume of air remaining in the lungs after a normal expiration.

8) **Total Lung Volume (TLV) or Total Lung Capacity (TLC)**: is the volume of air remaining in the lungs after a maximal inspiration. It is the sum of the vital capacity and residual volume.

9) **Dead space:**is the amount of air locked up in the air passages, i.e. the nasopharynx, trachea and bronchi.

10) **Minute Ventilation (MV) (pulmonary ventilation/Respiratory minutevolume):**Volume of air breathed in or out in one minute during normal respiration.

11) **Maximum Voluntary Ventilation (MVV):** is the volume of air which can be moved in or out of the lungs with maximum voluntary effort during one minute.

12) **Breathing reserve** = MVV-MV

13) **Breathing reserve percent or Dyspnoea Index = $\frac{\text{MVV – MV X 100}}{\text{MV}}$**

Normal value is 90-92% when it is reduced to 60-70% breathlessness or dyspnoe occurs in the individual.

14**)** **Ventilatory reserve = MVV/MV**

15) **Ventilatory equivalent:** This gives how many liters of air have to be ventilated for the absorption of 100ml of oxygen.

16) **Air Velocity index (AVI):** = $\frac{\text{\% predicted MVV}}{\text{\% predicted VC}}$

17) **Diffusing capacity of the lungs:** is the quantity of a gas that diffuses each minute for each mmHg difference in the partial pressure of this gas across the respiratory membrane.

Normal O_2 = 20ml/min/mm of Hg at rest.

CO_2 = 10-30ml/min/mm of Hg at rest.

Normal Respiratory values: (in human)

Tidal volume	- 500ml
IRV	- 2000-3000ml
ERV	- 1000ml
Residual volume	- 1200ml
Physiological dead space volume	- 150ml
Vital capacity	- 4.8ltr. Or 2.6 ltr/sq.m
Total Lung Capacity	- 6000ml

Predicted vital capacity	- Male – 2.5 ltr/sq.m
	Female – 2.0 ltr/sq.m
Rate of Respiration	- 14-18/minute.
Air velocity index	- 1
Ventilatory equivalent	- 2.75 lit (1.68-4.5 lit).

A modern USB PC-based spirometer

Screen for spirometry