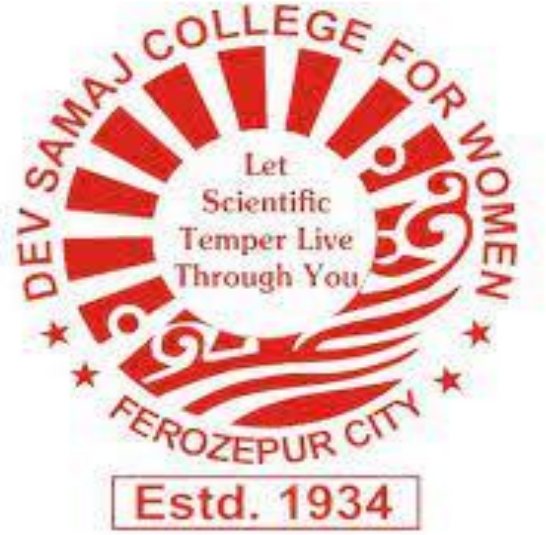
ZOOLOGY

LAB MANUAL

B.Sc. II



Department of Zoology

Dev Samaj College for women,
Ferozepur City, Punjab

Sr. No.	Experiment Name
1.	To estimate the Hb content of blood by using SAHLI'S Haemocytometer.
2.	To perform the activity of amylase in saliva and find the effect of different pH and temperature on enzyme activities
3.	To prepare the test & standard solution used in biochemistry lab and estimate glucose, protein, fat in given samples and also estimate urea, glucose, chloride and uric acid in the urine
4.	To estimation & calculation of the WBC, RBC, ESR of human blood by collecting the blood sample from peripheral and veins B.Sc. I

EXPERIMENT NO. 1

To estimate the Hb content of blood by using SAHLI'S Haemocytometer.

Sahli's/acid hematin Method

Principle: Blood is mixed with N/10 HCl resulting in the conversion of Hb to acid hematin which is brown in color. The solution is diluted till it's color matches with the brown colored glass of the comparator box. The concentrationof Hb is read directly.

Equipment required :

Hemocytometer consists :

- comparator box which has brown colored glass on either side
- Hb pipette which is marked upto $20mm^3$(0.02ml blood)
- Tube with markings of Hb on one side
- glass rod
- dropper

Reagents required

N/10 HCl

Distilled water

Sample: Venous blood collected in EDTA as described earlier

Procedure

1. Add N/10 HCl into the tube upto mark 2g%
2. Mix the EDTA sample by gentle inversion and fill the pipette with 0.02ml blood. Wipe the external surface of the pipette to remove any excess blood.
3. Add the blood into the tube containing HCl. Wash out the contents of the pipette by drawing in and blowing out the acid two to three times. Mix the blood with the acid thoroughly.
4. Allow to stand undisturbed for 10min.
5. Place the hemoglobinometer tube in the comparator and add distilled water to the solution drop by drop stirring with the glass rod till it's color matches with that of the comparator glass. While matching the color, the glass rod must be removed from the solution and held vertically in the tube.
6. Remove the stirrer and take the reading directly by noting the height of the diluted acid hematin and express in g%.

Fig. 3.1: Hb comparator box with brown glass on either side and tube with acid hematin solution in centre. The color of the solution is matched with the glass and the concentration of Hb is read directly

Advantages

- Easy to perform
- Quick
- Inexpensive
- Can be used as a bedside procedure
- Does not require technical expertise

Disadvantages

- Less accurate.
- All hemoglobins (oxyhemoglobin, sulphemoglobin) are not converted to acid hematin and hence the value of Hb obtained is less than the actual value.
- The color of acid hematin develops slowly.
- Color of acid hematin fades with time and dilution must be done exactly after 10 min when the color development is maximum
- Individual variation in matching of color is seen.

EXPERIMENT NO. 2

To perform the activity of amylase in saliva and find the effect of different pH and temperature on enzyme activities

Aim

A) To study the effects of variation in temperature and pH levels on the activity of salivary amylase on starch.

Necessary Materials & Apparatus

- Water.
- Ice cubes.
- Test tubes.
- Droppers.
- Wire gauze.
- Thermometer.
- Bunsen burner.
- Saliva solution.
- Iodine solution.
- pH tablets of 5, 6.7, 8.
- Beaker with water and a thermometer.
- 15 ml 1% starch solution + 3 ml 1% NaCl.
- 3 series of test tubes, each containing iodine solution.

Procedure

Effect of Various Temperatures on the activity of salivary amylase on starch

- Divide and pour the 15 ml 1% starch solution + 3 ml 1% NaCl solution into three test tubes and name them as A, B and C.
- Pour a few ice cubes in a beaker and ensure that they stay at 5 °C.
- Transfer tube- A to the beaker with ice.
- Take two more beakers and fill them with water.
- Heat the two beakers, one up to 37 °C and the other at 50 °C.
- Ensure that the temperatures for the two beakers are constant.
- Transfer test tube B into the beaker which is set at 37 °C.

- Similarly, transfer test tube C into the beaker set at 50 °C.
- Draw 1 ml of saliva solution and add it into test tube A. Do the same for test tube B and C.
- Quickly draw a few drops using a dropper from test tube A and transfer the same to the first series of test tubes having iodine solution.
- Repeat the same: transfer a few drops from test tube B and C into the second and third series of test tubes having iodine solutions.
- Note the time as "0-minute reading" and wait 2 minutes before proceeding to the next step.
- Draw a few drops from each tube and add it to the tubes with the iodine solution. Note the change in colour.
- Repeat the experiment in intervals of 2 minutes until the colour of iodine does not change.

Effect of different pH levels on the activity of salivary amylase on starch

- Divide and pour the 15 ml 1% starch solution + 3 ml 1% NaCl solution into three test tubes and name them as A, B and C.
- Add pH tablets 5, 6.8 and 8 into test tube A, B, and C respectively.
- Now add water into a beaker and boil it by placing it on a Bunsen burner.
- Transfer all the three test tubes into boiling water.
- Use a thermometer to ensure that the temperature of this water is to be maintained at 37 °C.
- Use a dropper to transfer 1ml of saliva solution to each of the three test tubes.
- Immediately transfer a few drops from test tube A to the first series test tubes containing iodine solution.
- Repeat the same for test tube B and C, transferring the same to series 2 and 3 test tubes respectively.
- Note the time as "0-minute reading" and wait 2 minutes before proceeding to the next step.
- Draw a few drops from each tube and add it to the tubes with the iodine solution.
- Note the change in colour.
- Repeat the experiment in intervals of 2 minutes until the colour of iodine does not change.

Observation

Effect of Various Temperatures on the activity of salivary amylase on starch: The test tube at 37 °C reaches the achromic point quickest compared to the other two. At high temperatures, the enzyme gets denatured and at low temperatures, the enzyme is deactivated. Hence, it takes more time for starch to be digested at temperatures outside 37° C.

b) To study Effect of different pH levels on the activity of salivary amylase on starch

The salivary amylase did not react in the tubes that had pH tablets of 5 and 8. It only reacted with the tube that had the pH tablet 6.8. The pH is considered acidic when it is level 5. A pH of 8 is considered to be alkaline. A pH of 6.8 s considered to be slightly acidic.

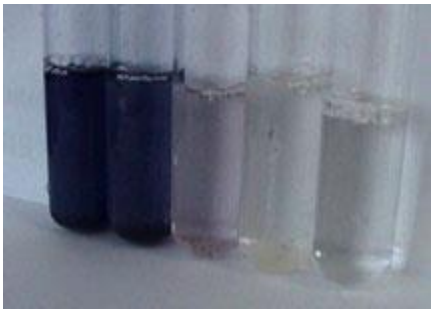


Fig 3.2: The effect of pH on Enzyme Activity – Salivary Amylase and Starch Digestion

EXPERIMENT NO. 3

To prepare the test & standard solution used in biochemistry lab and estimate glucose, protein, fat in given samples and also estimate urea, glucose, chloride and uric acid in the urine.

Theory

Nutrition is a source through which living entities obtain food for the body to get fueled with energy. Therefore the food we eat must be well-balanced with all the substances that are required by our body to perform daily activities, these substances are known as nutrients.

Nutrients nourish the body by releasing the energy required for the body's growth. Some of the nutrients found in food are:

- Proteins
- Carbohydrates
- Fats
- Vitamins
- Minerals
- Dietary fibres

What are proteins?

They are body-building nutrients which aid in the repair of damaged body parts and also in the growth of the body.

What are vitamins and minerals?

These nutrients are reflected to be protective food. They protect us from diseases by providing resistance against germs that are known to cause diseases.

What are carbohydrates?

Carbohydrates are nutrients that provide energy to the body instantly. Fats are also energy-giving foods and store energy.

Carbohydrates contain starch, sugars, and fibres which consist of sugar molecules containing hydrogen, carbon, and oxygen. Carbohydrates can be categorized into:

- Simple carbohydrates – They are composed of 1 or 2 units of sugar, hence can be broken down and absorbed easily. Simple carbohydrates are further divided into:
- Monosaccharides – Composed of one sugar unit

- Disaccharides – Consists of 2 chemically-associated monosaccharide units. Example – Lactose, Maltose, Sucrose, etc.
- Complex carbohydrates – These carbohydrates consist of long chains of simple carbohydrate units. Since they are large in size they can be broken down into simple carbohydrates. They are further subdivided into:
    - Oligosaccharides – Composed of less than 10 monosaccharides parts
    - Polysaccharides – composed of a large number of polysaccharides. Example – Glycogen, starch, and cellulose

Test for Sucrose

The presence of sucrose can be tested in a sample using Benedict's test.

Material Required

- Benedict's Reagent
- Sugar cane extract
- Concentrated HCl
- NaOH solution
- Burner
- Dropper
- Test tube
- Test tube holder

Procedure

- Take a clean and dried test tube and add sugar cane extract into it.
- Now carefully add a few drops of concentrated HCl using a dropper to the test tube.
- Hold the test tube securely with the help of a test tube holder.
- Place the test tube near the Bunsen burner and allow the solution to boil for two minutes.
- While boiling, the hydrolysis of sucrose occurs and the fructose converts to glucose.
- With the help of a dropper, add a few drops of NaOH solution to the test tube so that the solution turns alkaline.
- Now add a few drops of Benedict's reagent with the help of a dropper into the test tube.
- With the help of a test tube holder, place the test tube near the Bunsen burner and allow the solution to boil for a few minutes.
- Observe the changes.

Observation

The colour of the solution colour from blue colour to green colour. From green colour, it finally changes to brick red or orange colour. This indicates that the solution contains glucose.

Test for Starch

Material Required

- Potato extract
- Iodine solution
- Test tube
- Dropper

Procedure

- Take a clean and dried test tube and add potato extract into it.
- Add five to six drops of iodine solution with the help of a dropper into the test tube.
- Keep the test tube undisturbed and allow the mixtures to stand for a few seconds.
- Observe the changes.

Observation

The presence of starch in the potato extract is indicated when the colour changes to a blue-black colour.

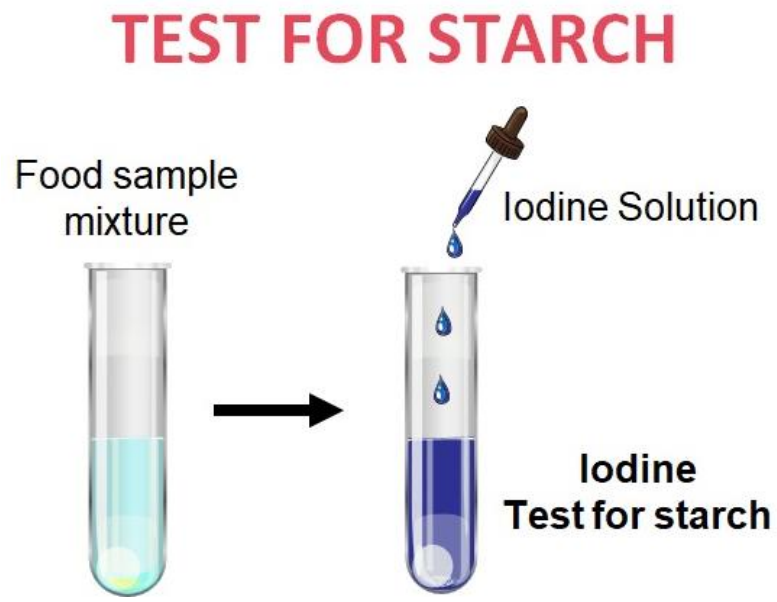


Fig 3.3: Test for Starch

Test for Proteins

The presence of proteins in a sample can be detected by the following tests:

- Biuret Test
- Xanthoproteic Test
- Million's Test

1. Biuret Test

Material Required

- 1% $CuSO_4$
- 40% NaOH solution
- Dropper
- Egg Albumin
- Test tube
- Test tube holder

Procedure

- Take a clean and dried test tube and add egg albumin into it.
- Now add a few drops of 40% NaOH solution with the help of a dropper into the test tube containing the egg albumin.
- With the help of a dropper, add 2 to 3 drops of 1% $CuSO_4$ solution into the same test tube contained in the egg albumin.
- Now shake the test tube slowly in order to mix the solution present in it completely.
- Keep the test tube undisturbed and allow the mixtures to stand for 5 minutes. After a few minutes, observe the changes.

Observation

The solution in the test tube appears to be violet in colour. This indicates that the sample that is tested contains proteins.

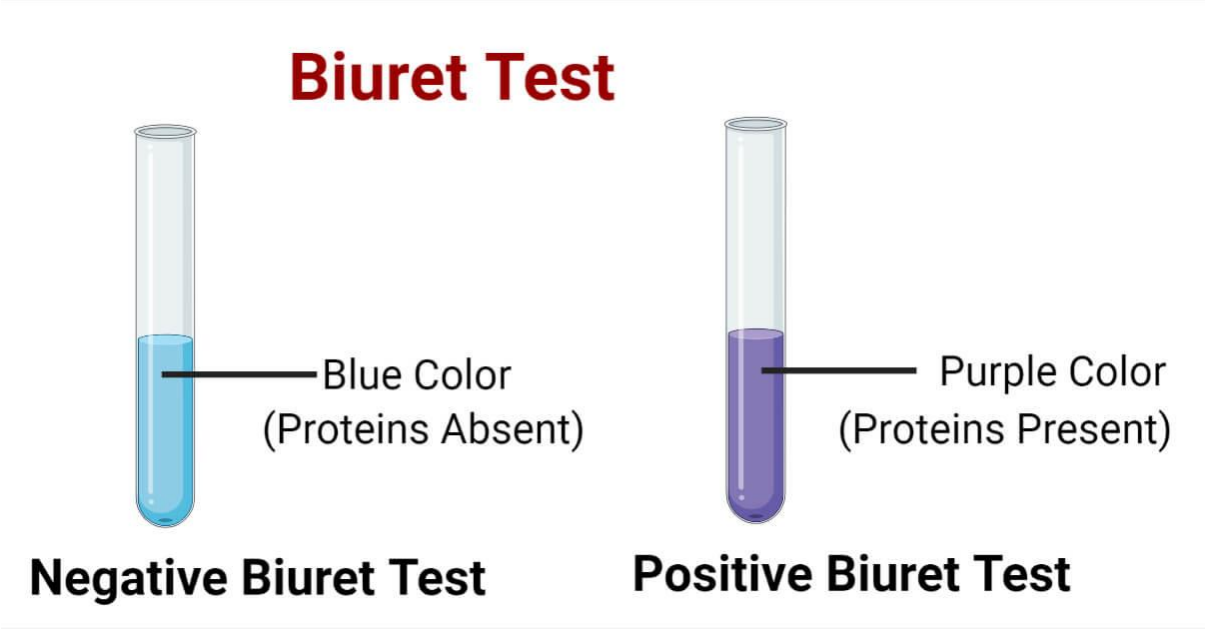


Fig 3.4: Biuret test for proteins

2. Xanthoproteic Test

Material Required

- Ammonia solution
- Concentrated HNO3
- Dropper
- Test tube
- Egg albumin
- Test tube holder

Procedure

- Take a clean and dried test tube and add egg albumin into it.
- Add five drops of concentrated HNO3 with the help of a dropper into the test tube containing the egg albumin.
- Holding the test tube securely with a test tube holder, bring the sample to a boil over a burner.
- As the solution starts to boil, a yellow precipitate is formed.
- Now add a few drops of ammonia solution into the test tube slowly.

- Stir the test tube continuously in order to mix the solution completely. After a few minutes, observe the changes.

Observation

The sample contained in the test tube, which appeared to have yellow precipitate changed its colour to orange. This indicates that the sample contains proteins.

3. Million's Test

Materials Required

- Million's Reagent
- Egg albumin
- Test tube
- Dropper

Procedure

- Take a clean and dried test tube and add egg albumin into it.
- With the help of a dropper, add a few drops of Million's reagent into the test tube containing the egg albumin.
- Keep the test tube undisturbed and allow the mixtures to stand for 5 minutes.
- After a few minutes, observe the changes.

Observation

The sample indicates the presence of proteins when the colour changes to pink.

Test for Fats

The presence of fats in a sample can be detected by the following tests:

- Sudan III Test
- Paper Spot Test

1. Sudan III Test

Materials Required

- Sudan III Solution

- Oil
- Test tube
- Dropper
- Egg Albumin

Procedure

- Take a clean and dried test tube and add a few drops of oil into it.
- Now into the same test tube, add five to six drops of the Sudan III reagent with the help of a dropper.
- Stir the test tube continuously and allow the solution to stand for a while.
- After a few seconds, observe the changes.

Observation

The presence of fat in the sample is indicated when pink colour droplets appear on the test tube.

2. Paper Spot Test

Materials Required

- A piece of white paper
- Peanut seeds
- Watch glass

Procedure

- On the piece of white paper, place the fresh peanut seeds.
- Crush and rub the peanut seeds on the white paper.
- Remove the peanut seed remaining on the watch glass.
- Observe the changes on the piece of white paper.

Observation

The spot where the peanut seeds are rubbed turns translucent. This indicates the presence of fats in the sample.

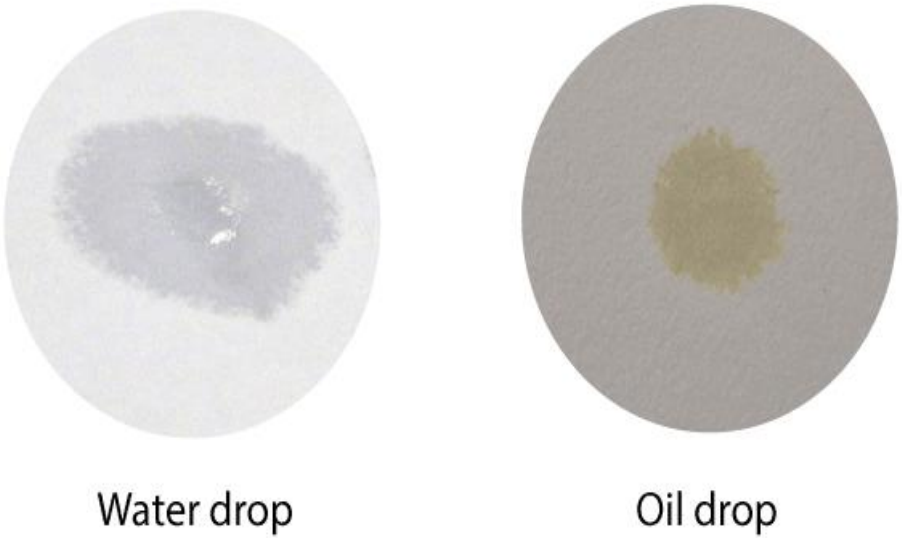


Fig: 3.5 Paper spot test for fats

Aim

To perform a test detecting the presence of urea in the given sample of urine.

Theory

What is Urine? Describe human urine.

Urine is produced by the kidney. It is a liquid waste that needs to be eliminated from the body. The kidneys eliminate the wastes from the bloodstream. In humans, urine is a yellowish liquid containing water and several other chemical components such as uric acid, urea, traces of enzymes, hormones, and carbohydrates.

Production of Urea

It is produced naturally by the body while the proteins are disintegrated during which amino groups are eliminated from the amino acids that are in the proteins. The eliminated amino groups are converted into extremely toxic ammonia and furthermore into urea by the liver. The urea moves into the kidneys, where it is finally eliminated from the body.

Detecting urea

For detection of urea in the urine sample, urease tests are performed. Urease is an enzyme that carries out the decomposition of urea into carbon dioxide and ammonia. Addition of an alkaline substance such as ammonium carbonate causes it to turn into an alkaline solution from slightly acidic urine.

1. Sodium Hypobromite Test

Material Required

- Dropper.
- Test tube.
- Urine sample.
- Measuring cylinder.
- Sodium hypobromite solution.

Procedure

- Take a clean and dried test tube.
- With the help of a measuring cylinder, add 2ml urine sample into the test tube.
- To the same test-tube add 2 to 3 drops of sodium hypobromite solution using a dropper.
- Mix the solution well.
- Observe the changes.

Observation And Conclusion

The presence of urea in the given urine sample is indicated by the presence of brisk effervescence of nitrogen around the test tube.

2. Urease Test

Material Required

- Spatula.
- Dropper.
- Urine sample.
- 1% acetic acid.
- 2% of Na_2CO_3.

- Urease powder.
- Measuring cylinder.
- Phenol red indicator.

Procedure

- Take a clean and dried test tube.
- With the help of a measuring cylinder, measure and add 5ml urine sample into the test tube.
- To the sample test tube, add 4 to 5 drops of the phenol red indicator using a dropper.
- With the help of another dropper, add drops of 2% Na_2CO_3 solution drop-wise until the pink colour starts developing in the test tube.
- With the help of another dropper, add a few drops of 1% acetic acid. Add drop-wise until the pink colour disappears.
- With the help of a spatula, add little urease powder into the urine sample test tube.
- Now shake the test tube slowly until the urease powder get mixed.
- Now observe the changes.

Observation And Conclusion

The existence of urea in the given sample of urine is indicated when a red or pink colour appears in the solution.

Fig 3.6: Urease Test

Aim

To perform a test detecting the presence of sugar in the given sample of urine.

Theory

Urine is an excretory liquid waste, produced by a pair of a kidney, which needs to be eliminated from our body. Through the process of urination, urine is excreted from the urethra. In humans, urine is a pale yellowish liquid containing water and several other chemical components such as uric acid, urea, traces of enzymes, hormones, and carbohydrates.

Properties Of Urine

- It has a characteristic pale yellow colour.
- The yellow colour is imparted by the yellow pigment known as urochrome.
- The urine pH ranges from 4.6 to 8.
- More than 95% of urine constitutes water.
- Organic substances of nitrogenous origins found in urine are creatine, uric acid, urea.
- Other organic matter in urine are lactic acid, oxalic acid.
- Inorganic constituents are potassium chloride, sodium chloride, phosphates, and sulphates.
- Urine abnormally can contain other constituents such as ketone bodies, sugar(glucose), protein, bile and blood.
- Glucose is usually not found in urine. It appears when in blood, the glucose levels exceed the renal threshold of glucose i.e., 160 to 180 mg/dl.
- When glucose is present in urine it is termed as glucosuria. It indicates diabetes mellitus.

Also Read: Diabetes mellitus.

Sugar presence in a sample of urine can be detected by performing the following two tests:

1. Benedict's test
2. Fehling's test

What is Benedict's Test?

A Benedict's solution serves as a reagent in this test. The reagent is a blend of copper, sodium citrate and sodium carbonate and copper II sulphate pentahydrate ($CuSO_4.5H_2O$)

What is Fehling's test?

In this test, the two different types of Fehling's solution are used:

1. Fehling's solution – A: Aqueous solution of copper II sulphate – Blue colour solution.
2. Fehling's solution – B: Aqueous solution of sodium potassium tartrate – Clear and Colourless solution.

When the urine sample is boiled with the two different reagents, the $CuSO_4$ found in Benedict's and Fehling's solution is reduced by the reducing agent, glucose for the formation of a coloured cuprous

oxide precipitate. Depending on the glucose concentration, a yellow, green and brick-red formation of precipitates of oxides take place.

1. Benedict's Test

Material Required

- Burner.
- Test tube.
- Urine sample.
- Test tube holder.
- Benedict's solution.
- Measuring cylinders.

Procedure

- Take a clean and dried test tube.
- Using the measuring cylinder, accurately measure 2ml of the given urine sample.
- Pour the measured urine sample into the test tube.
- Add accurately 5ml of Benedict's reagent into the test tube containing the urine sample.
- Now fix the test tube holder, bring the test tube near the bunsen burner and allow it to heat for 2 minutes.
- While it is heating, keep stirring the tube continuously.
- Observe the changes.

Observation And Conclusion

Upon heating the sample, gradually, a yellow precipitate is formed in the test tube, which indicates the presence of sugar in the given urine sample. Different precipitates are formed depending upon the sugar concentration in urine, which can be yellow, green, or brick red.

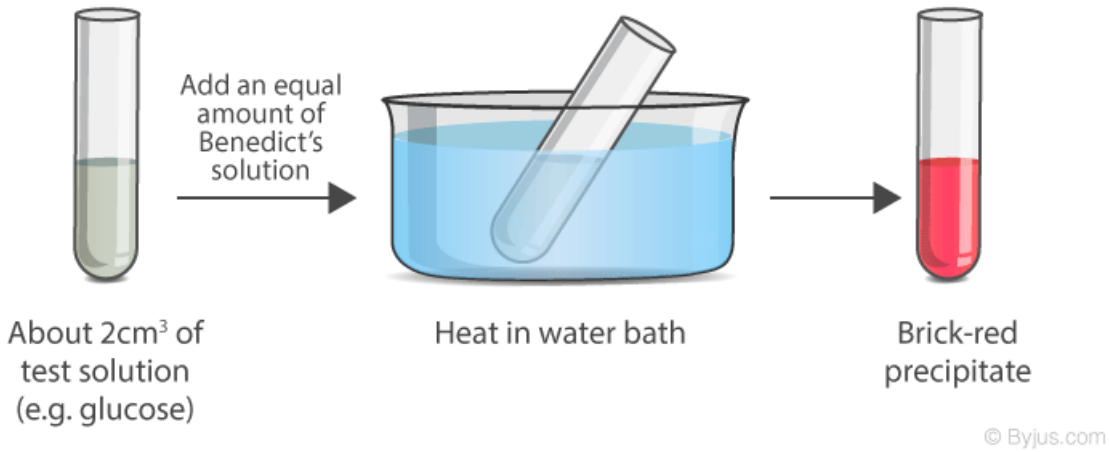


Fig 3.7: Benedict's test

2. Fehling's Test

Material Required

- Burner.
- Test tube.
- Urine sample.
- Test tube holder.
- Measuring cylinder.
- Fehling's solution A.
- Fehling's solution B.

Procedure

- Take a clean and dried test tube.
- Using the measuring cylinder, accurately measure 2ml of the given urine sample.
- Pour the measured urine sample into the test tube.
- Add accurately 2ml of Fehling's solution A into the tube containing urine sample and shake well.
- Add accurately 2ml of Fehling's solution B into the same test tube and mix all the solution slowly.

- Now fix the test tube holder, bring the test tube near the bunsen burner and allow it to heat for 2 minutes.
- While it is heating, keep stirring the tube continuously
- Notice the changes

Observation And Conclusion

Upon heating the sample, gradually, a green precipitate is formed in the test tube, which indicates the presence of sugar in the given urine sample. Different precipitates are formed depending upon the sugar concentration in urine which can be yellow, green, brick red.

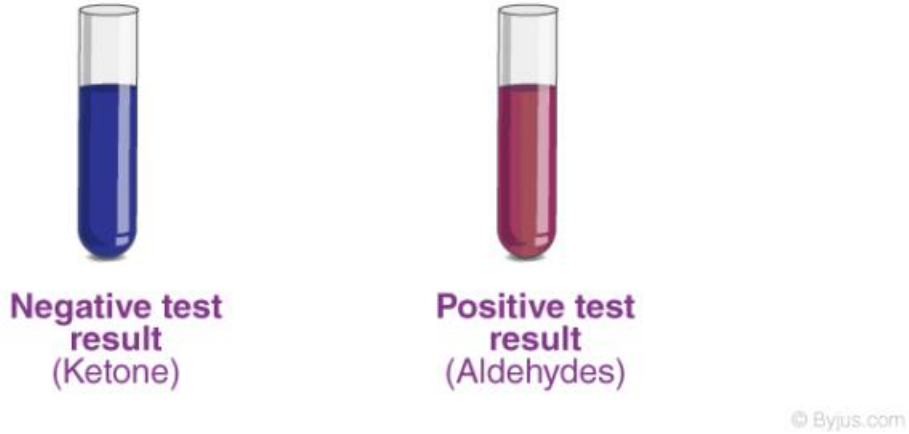


Fig 3.8: Fehling's test

EXPERIMENT N0. 4

To estimation & calculation of the WBC, RBC, ESR of human blood by collecting the blood sample from peripheral and veins

a) To estimation & calculation of the WBC

Background: White blood cell count is an enumeration of white corpuscles or is an leucocyte count. The white blood cells (WBCS)or leucocytes are nucleated actively amoeboid and do not contain haemoglobin and ae originated purely from extravascular tissue. They are composed of nucleoproteins and varieties of enzymes. Their number is less and life span is short as compared to red blood cells. The WBCs exist in two forms viz. granulocytes and agranulocytes. Granulocytes are further classified as eosinophil, basophil, neutrophil, while agranulocytes shows lymphocytes and monocytes. These varieties possess independent morphological, functional and staining properties. The main function of white blood corpuscles is phagocytosis that is body defence mechanism against foreign particles and invading bacteria. They are also involved in antibody formation in immunological body defence mechanism. It also take part in process of repair in an area of inflammation. The basic principle is that the blood is diluted with acid solution which removes the red cells by haemolysis and also accentuates the nuclei of the white cells; thus the counting of the white cells becomes easy. Blood cell counts can be performed using the hemacytometer.

Significance: The white cell count is the number of white cell present in one cubic millimeter of blood. The normal values of white blood cell count varies between 5000 to 10,000 per cubic millimeter or 7-11 thousand cells/µl of blood volume in healthy individual. Variation in normal values is observed in diseased states. WBC count increases (leucocytosis) in conditions like pneumonia, leukemia, meningitis, small pox etc. while the count decreases (leucopenia) in conditions such as influenza, typhoid, infectious hepatitis etc. Moreover the count rises in pregnancy and during menstruation. Thus, white blood cell count is useful in diagnosis. The aim of the experiment is to estimate white blood cell count of a blood specimen.

REQUIREMENTS: Neubaur chamber, WBC pipette, Cover slip, WBC diluting fluid, Needle, spirit, cotton.

PROCEDURE:

1. Sterilize the finger tip with cotton plug soaked in 70% alcohol and let it dry.

2. Take a bold prick to have free flow of blood and draw the blood in a WBC pipette up to 0.5 mark.

3. Dip the WBC pipette in WBC diluting fluid up to 11 mark and rotate the pipette equally in your hands to mix the solution well by swirling.

4. Take the haemocytometer and place it on the flat surface of the work bench. Place the cover slip on the counting chamber.

5. Allow a small drop of diluted blood, hanging from the pipette, to sweep into the counting chamber by capillary action. Make sure that there is no air bubble and there is no overfilling beyond the ruled area.

6. Leave the counting chamber on the bench for 3 minutes to allow the cells to settle. Observe the cells by placing the counting chamber on the mechanical stage of the microscope.

Focus on one of the corner squares of the counting chamber and count the white cells schematically, starting from the upper left small square of each Square. Repeat the count in all the four corners of the chamber. Apply the margin rules i.e. count the cells lying on two adjacent margins, and discard those on the other two margins.

DATA ANALYSIS:

No. of cells X Dilution factor X Depth factor/ Area count

Where

Dilution factor = 20, Depth factor = 10, Area count = 4

RESULT: The number of white blood cells present in one µl of blood specimen is_____

b) ESTIMATION OF TOTAL RED BLOOD CORPUSCLES (RBC) COUNT

Background: Red blood cell count is an enumeration of red cells or is any erythrocyte count.

The red bllod cells or erythrocytes are circular, biconcave, non nucleated cells containing haemoglobin and are embeded in blood plasma. After birth bone marrow is the main site of formation of red blood corpuscles. These are involved in acting as a carrier of oxygen and carbon dioxide. RBCs also maintain the ionic balance of human physiological system and maintains viscosity of blood. Various pigments like bilirubin and biliverdin are derived from RBC after their degradation.

The basic principle is that the blood specimen is diluted (usually 200 times) with red cell diluting fluid which does not remove the white blood cells but allows the red cells to be counted under magnification in a known volume of fluid. Finally, the number of cells in undiluted blood is calculated and reported as the number of red cells/µl of whole blood.

Blood cell counts can be performed using the hemacytometer.

Significance: The red cell count is the number of red cells present in one cubic millimeter of blood.

The normal values of the red blood cell count are:

Woman : 4-5.5 million per cubic millimeter

Men : 4.5-6.0 million per cubic millimeter

Infants : 5- 6.5 million per cubic millimeter

Variations in normal values is observed in pregnancy, severe burns, diseased conditions and it also depends upon altitude. It drops below normal values in anaemia and leukemia and rises above the normal values in polycythemia and dehydration conditions. Therefore, the red cell count is useful in diagnosis.

The aim of the experiment is to estimate red blood cell count of a blood specimen.

REQUIREMENTS: Neubaur chamber, RBC pipette, Cover slip, RBC diluting fluid, Needle, spirit, cotton.

PROCEDURE:

1. Sterilise the finger tip with cotton plug soaked in spirit and let it dry.

2. Take a bold prick with needle to have free flow of blood and draw the blood in a RBC pipette upto 0.5 mark.

3. Dip the RBC pipette in red blood cell diluting fluid and suck up diluting fluid upto 101 mark.

4. Rotate the pipette equally in your hands to mix the solution well by swirling.

5. Take the haemocytometer and place it on the flat surface of the work bench. Place the cover slip on the counting chamber.

6. Allow a small drop of diluted blood, hanging from the pipette, to sweep into the counting chamber by capillary action. Make sure that there is no air bubble and the counting chamber must not be flooded

7. Leave the counting chamber on the bench for 3 minutes to allow the cells to settle. Observe the cells by placing the counting chamber on the mechanical stage of the microscope.

8. Focus on the centre room of the chamber and start counting the cells from upper left corner of the room. It is advisable to complete all counts of the four squares and then move to the centre square, which is the fifth square to be counted.

DATA ANALYSIS:

No. of cells X Dilution factor X Depth factor X Total ruled area/Area count

Where;

Dilution factor = 200; Depth factor = 10; Total ruled area = 25; Area count = 5

RESULT: The number of red blood cells present in one µl of blood sample is_____

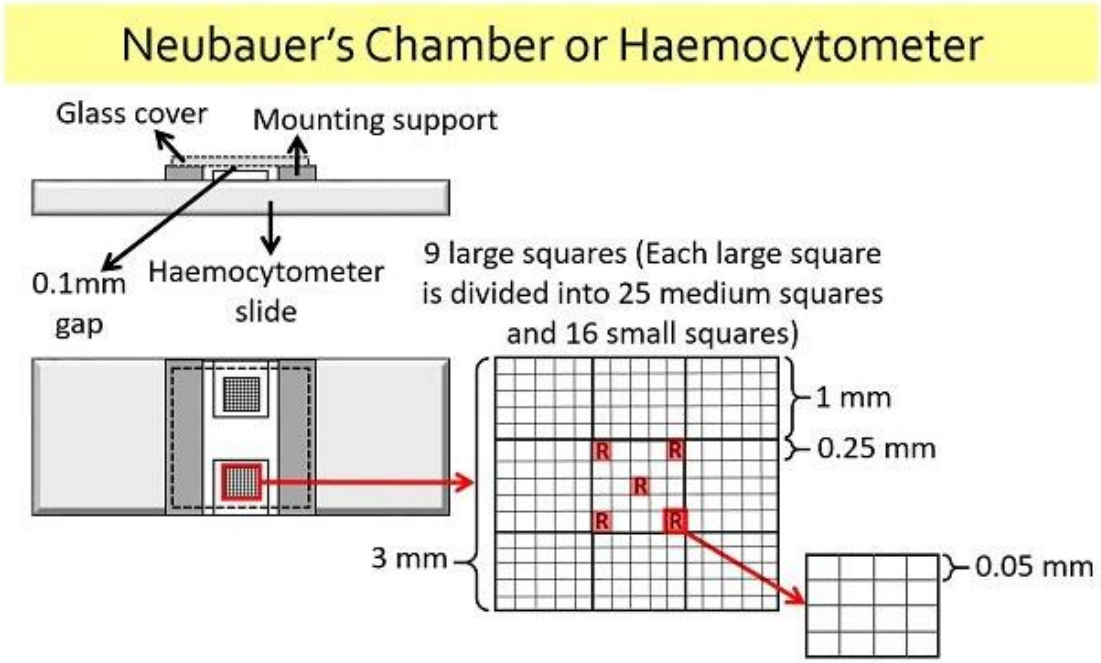


Fig 3.9: Neubaur chamber

c) Erythrocyte sedimentation rate (ESR): principle, method, procedure and clinical application

Principle of ESR:

When an anticoagulant is added to the blood and this well mixed venous blood is placed in a vertical tube, erythrocytes tend to settle towards bottom leaving clear plasma on top. This rate of sedimentation of red blood cells in a given interval of time is called erythrocyte sedimentation rate (ESR).

As the erythrocytes sediments, in a period of one hours, 3 stages can be observed.

- **Stage I: first 10 minutes**
  - It is initial period of aggregation during which rouleaux are formed and the sediment rate is low
- **Stage II: next 4o minutes**
  - It is a period of fast setting. Sedimentation occurs at a constant rate during this period
- **Stage III: next 10 minute or more**

- The sedimentation again slows as it is the final period of packing of cells at the bottom of the tube

Factors affecting ESR:

- There are several factors that affects sedimentation of erythrocytes.
- **Factors that increases ESR:**
  - **Anemia:**
    - anemia increase ESR because the change in erythrocyte-plasma ratio favors rouleaux formation.
    - Rouleaux is aggregation of RBCs together due to their discoid shape.
    - Rouleaux have a decrease surface area and accelerate ESR
  - **Increase level of fibrinogen:**
    - it decreases the negative charge of erythrocyte, so RBC tend to remain apart and this promotes formation of rouleaux and increase ESR
  - **Immunoglobulin:**
    - increase antibody level in blood increase ESR
  - **Increase cholesterol level**
  - **Rheumatoid arthritis**
  - **Chronic infections**
  - **Carcinoma**
  - **Tissue destruction and other disease**

- **Factors that decrease ESR:**
  - **Defibrinigenation:**
    - removal of fibrinogen decreases ESR
  - **Increase albumin** and **lecithin** in blood
  - **Abnormal** or **sickle shape RBCs**:
  - abnormal or irregular shape of RBC lower ESR
  - **Congestive heart failure**

Method for ESR estimation:

Westergren method for ESR estimation is widely used method. Wintrobe method is also used for ESR determination. Wintrobe tube is smaller than westergren tube

Materials required:

1. Westergren tube or wintrobe tube
2. Anticoagulant: 0.1 M sodium citrate
3. ** in modified westergren method EDTA is used as anticoagulant

Procedure for ESR estimation:

- Withdraw 4 ml of venous blood
- Mix exact 10ml of sodium citrate with 4ml of venous blood in a tube
- Invert the tube 2-3 times to mix the blood thoroughly with anticoagulant
- Fill the westergren tube up to mark 0 and place in the rack at room temperature undisturbed and away from sunlight.
- Take the reading exactly after 1 hour. Record in millimeters from top surface of column to top of RBC sediments.

Result:

- Normal value of ESR
    - Female:
        - under 50 years- 20 mm/hr
        - above 50 years- 30mm/hr
    - Male:
        - Under 50 years- 15mm/hr
        - Above 50 years- 20 mm/hr

Clinical application of ESR estimation:

- ESR test is non-specific test although it is used as indication of presence of disease
- ESR value increase during rheumatoid arthritis, chronic infection, carcinoma, tissue destruction and nephritis
- During pregnancy, ESR increase moderately from 10^{th} or 12^{th} weeks onwards and return to normal after delivery.
- ESR value decreases in sickle cell anemia and congestive heart failure (CHF).

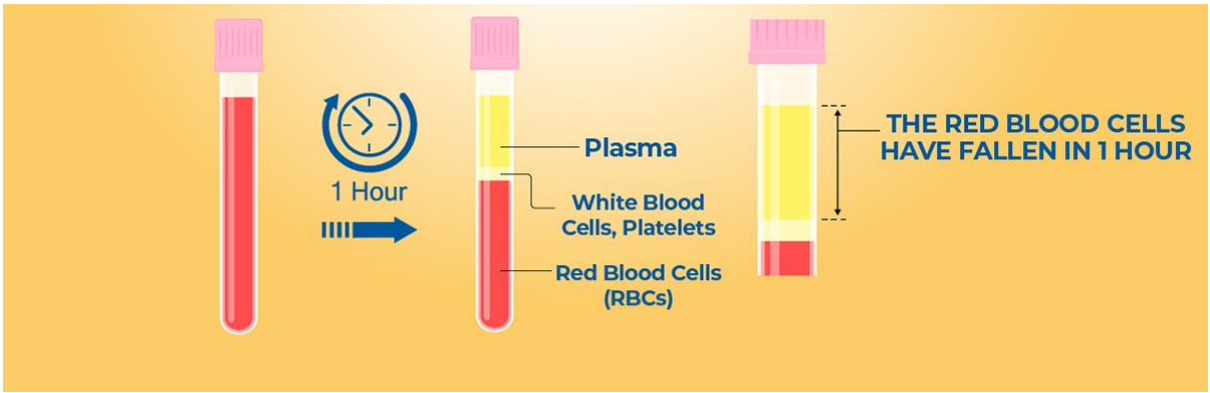


Fig 3.10: ESR Estimation