carefully to another tube for use. Ideally about50 percent serum is obtained from the blood volume. The serum is kept in a cold place or may be frozen if the analysis is delayed. Serum is similar to plasma in composition but there is no fibrinogen and also a diminished concentration of other clotting factors such as prothrombin. **(1) Normal serum** - It is clear and light yellow in appearance. **(II) Haemolysed serum - Reddish in colour, haemolysed serum is not suitable for analysis. (III) Icteric serum - Golden yellow in colour; found in jaundiced cases due to increased billrubin. (IV) Lipaemic serum -Milky in appearance due to excess of lipids in blood. It is also some times found when blood is drawn just after a rich fat diet.**

Specimen labelling - After collecting the specimens under prescribed conditions the sample vials should be labelled with the name of the patients, ward/bed and the name of the test to be done. The sample then be sent to the laboratory as soon as possible invariably accompained by a report slip with the details as labelled on the sample vial and also the clinical impression. This practice prevents the loss or misplacement of samples and also enables the analyst to assess the reliability of the results on the basis of clinical diagnosis mentioned. **If the sample can not be sent soon for the analysis, it should be properly kept in a cool place, preferably in a referigerator at 4^{o}C.**

Anticoagulants - When whole blood or plasma is required in a test, the specimen is collected in a glass vial containing certain substances which have the property of preventing the clotting of blood. These substances are called anticoagulants. The following ones are in common use:

1. Heparin- This is the most satisfactory anticoagulant because its use produces no change in the composition of the blood. But it is expensive, so not used commonly. Heparin inhibits the formation of thrombin from prothrombin and thus prevents clotting **(i) Sodium heparin-** 0.1 ml of 1 percent aqueous solution of sodium heparin (100 units per mg) is sufficient for about 10 ml blood **(ii) Calcium heparin-** 2 mg per 10 ml blood is used; 0.2 ml of one percent aqueous solution (50 units per mg) **(iii) Lithium heparin-** 2 mg per 10 ml blood.

2. Potassium oxalate (single oxalate vial)- It is usually used in biochemical tests when whole blood or plasma is required in the test. Potassium oxalate is most commonly used as it is more soluble than sodium oxalate. The anticoagulant property of oxalate salts is due to the fact that they precipitate the calcium ions of the blood as calcium oxalate and so the calcium ions which are necessary for initiating clotting of blood are not available.

Three mg per ml blood is sufficient. 3 g of potassium oxalate is dissolved in 100 ml distilled water; 0.1 ml (2 drops) is added to each vial and dried in electric oven below 100 ^{0}C. It is suitable for 1 ml blood.

3. Potassium oxalate - Ammonium oxalate (double oxalate vial)- This is mostly used in haematological investigations such as TLC, DLC, Hb etc. It is cheaper and most .commonly used. This combination does not affect the composition of the blood. Potassium oxalate slightly shrinks the cells while ammonium oxalate swells the cells, as a result the combination is able to maintain the morphology of the cells.

2 to 3 mg of the mixture is satisfactory for 1 ml blood (2 parts potassium oxalate and 3 parts ammonium oxalate). Dissolve 4g potassium oxalate and 6 g ammonium oxalate in 100 ml water. Add 0.1 ml (2 drops) in each vial and dry below 100 ^{0}C in oven. The vial is adequate for about 5 ml of blood.

4. Potassium oxalate-sodium fluoride (fluoride vial)- This combination is used in the collection of blood and other body fluids only for the determination of glucose by chemical methods. This is not used for glucose estimation by enzymatic methods such as glucose oxidase method because fluoride is enzyme poison. **Potassium oxalate acts as an anticoagulant and fluoride as antiglycolytic agent (i.e. prevents conversion of glucose to lactic acid).**

Six mg potassium oxalate and 2 mg sodium fluoride is satisfactory for 1 ml blood. 6 g potassium oxalate and 2 g sodium fluoride is dissolved in 100 ml water. 0.1 ml (2 drops) is added to each vial and dried in oven below 100 ^{0}C. The vial is adequate for 1 ml of blood sample.

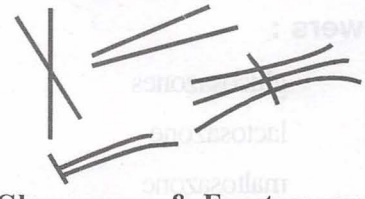
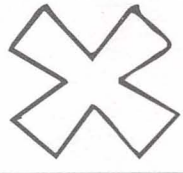
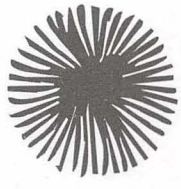
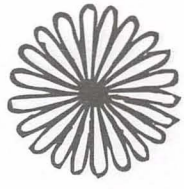
5. Ethylene diamine tetra acetic acid (EDTA)- It is used in some haematological investigations, e.g. platelet counts. Dipotassium salt of EDTA is preferred to sodium salt because of its greater solubility. EDTA prevents clotting by chelating calcium ions; 10-20 mg per ml blood is satisfactory.

6. Sodium citrate- This is also used in haematological tests such as prothrombin time and ESR. Citrate does not precipitate the calcium but converts it into a non-ionizable form and prevents clotting. 5-6 mg per ml blood should be used. Appreciable withdrawal of water from the cells results.

PRACTICAL **3**

FORMATION OF OSAZONES

(Glucose, Fructose, Galactose, Lactose, Maltose and Sucrose)

TEST	OBSERVATION	INFERENCE
5 ml. sugar solution + 10 drops glacial acetic acid + pinch of phenylhydrazine hydrochloride + twice the amount of sodium acetate crystals, mix and warm to dissolve the solids, filter and keep the filtrate in boling water bath for 20 minutes.		
1. Glucose solution (0.5%)	Glucosazone formed which are **broomstick like or needle shaped** (minimum time of formation of crystals is 5 minutes).	
2. Fructose solution (0.5%)	Glucosazone formed which are **broomstick like or needle shaped** (minimum time of formation of crystals is 2 minutes).	 **Glucosazone & Fructosazone** form same type of osazones
3. Galactose solution (0.5%)	Galactosazone formed which are **Rhombic like** (minimum time of formation of crystals is 7 minutes).	 **Galactosazone**
4. Lactose solution (2%)	Lactosazone formed which are like **ball of prickles or powderpuff like** (minimum time of formation of crystals is 10-12 minutes).	 **Lactosazone**
5. Maltose solution (2%)	Maltosazone formed which are **sunflower shaped** (minimum time of formation of crystals is 10-15 minutes).	 **Maltosazone**
6. Sucrose solution (2%)	Osazone not formed	Sucrose does not form osazone

TEST FOR UROBILINOGEN

TEST	OBSERVATION	INFERENCE
Ehrlich's Test 5 ml urine + 5 ml Ehrilich's reagent. Wait for 10 minutes, then add 10 ml saturated sodium acetate	Pink or red colour develops	Urobilinogen present

TEST FOR PENTOSES

TEST	OBSERVATION	INFERENCE
Bial's test for pentoses. 5 ml Bial's reagent + 0.5 ml urine, mix. Heat to 100°C for 30 seconds. Allow to stand for 5-20 minutes	Green/blue colour is obtained	Pentoses present

TEST FOR BENCE-JONES PROTEIN

Bence - Jones protein may be distinguished from other proteins found in urine, **as it will precipitate at temperatures between 40-60^{0}C,** whereas other urine proteins (derived from plasma proteins) precipitate between 60-70 ^{0}C. On raising the temperature higher than 60^{0}C, Bence - Jones protein redissolves, but other proteins do not. On cooling to 60^{0}C from boiling, Bence - Jones protein re-precipitates. Bence - Jones protein gives a positive reaction with the sulphosalicylic acid test given above, so this forms a useful screening test. If this was negative, there is, therefore, no point in proceeding to the heating test given below.

It is a non-blood protein. It is believed to be of diagnostic importance in cases of multiple myeloma and myelogenic osteosarcoma.

1. Filter urine, and adjust reaction until just acidic.
2. Place 5 ml of this urine into each of three tubes.

   Acidify one of these tubes with one drop of 33% acetic acid, and another with two drops of acid.
3. Place a thermometer in the urine and heat the three tubes in a beaker of water.
4. Carefully observe the temperature and any signs of precipitation.
5. Continue heating until the urine boils.

Note: If protein other than Bence - Jones is present, allow the urine to boil, filter off this precipitate while still hot, and test the clear filtrate as above.

Fill in the blanks/MCQs/Questions/Answers :

Q 1. Urine is normally pale yellow in colour due to the presence of pigment .....

Q 2. pH of normal urine is about .....

Q 3. Normal constituents of urine are ..... in number.

Q 4. Specific gravity of a normal urine is generally in the range of ..... to .....

Q 5. Specific gravity of urine, in diabetes mellitus may be as high as .....due to high concentration of .....

S. No.	EXPERIMENT	OBSERVATION	INFERENCE