BIO 303

Biochemistry – II

LABORATORY MANUAL

DEPARTMENT OF BIOLOGY

VIRTUAL UNIVERSITY OF PAKISTAN

LAHORE

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Contents

Experiment No. 1

Preparation of stock and working solutions in Laboratory

Apparatus Required

Weighing balance, volumetric flask, Cylinder, beaker, stirrer, pH meter or litmus paper

Chemicals required

Sodium chloride, Hydrochloric acid, sulfuric acid

Theory

Solution:

"A solution is a homogenous mixture of atoms, ions or molecules of two or more substances."
A homogenous mixture is that which has uniform position throughout its body.

Solvent and Solute

The solvent is the component of a solution that is visualized as dissolving another component called absolute. Usually the component present in the larger quantity is called the solvent, and the component present in the smaller quantity is called the solute.

Types of solution

Unsaturated solution

A solution that is capable of dissolving more solute at a given temperature than it already contains, is known as unsaturated solution.

Saturated solution

A saturated solution is the solution which can dissolve no more amount of the solute, at a given temperature.

Super saturated solution

A solution that contains more dissolved solute than a saturated solution is called super saturated solution.

Concentration and its units

Concentration means the relative amounts of the components of a solution. It tells the ratio of the quantity of one component to the quantity of the other or to the total quantity of solution. It has many units. Some common units are discussed below.

Mass Percentage

The ratio of the mass of the solute to the mass of the solution multiplied by 100 is called mass

percentage.

Mass Percentage of a solute = $\frac{\text{Mass of the solute} \times 100}{\text{Mass of solution}}$

For liquid-liquid solutions, it is sometimes more convenient to express the concentration in the units of percentage by volume:

Volume Percentage of a liquid = $\frac{\text{Volume of the liquid} \times 100}{\text{Total volume}}$

Parts per Million (ppm)

This is used to express very dilute concentrations of a substance. One ppm is equal to 1mg of solute dissolved per litre of solvent or 1mg of solute dissolved per kg of solvent.

Molarity (M)

Molarity or the molar concentration is the number of moles of solute dissolved per dm^3 of solution. (1 dm^3 is equal to 1Liter)

Molarity = $\frac{\text{Number of moles of solute}}{\text{Volume of solution in liters}}$

Molality (m)

Molality is the number of moles of solute dissolved per kilogram of solvent.

Normality (N)

Normality is the number of equivalents dissolved per liter of solution.

$$\text{Normality} = \frac{\text{No. of equivalents}}{\text{Volume of solution in Litres}}$$

pH

The pH is defined as follows:

"The pH of a solution is the negative logarithm to the base 10 of the hydrogen ion (or Hydronium ion) concentration".

$pH = -\log_{10}[H^+]$

$pH = -\log_{10}[H3O^+]$

The pH of a neutral solution is 7, the pH of acids is less than 7 while that of bases is higher than

7.

Procedure

i. Preparation of 5% NaCl solution

1. Weigh 5grams of sodium chloride.
2. Dissolve the sodium chloride (NaCl) in 25 ml of distilled water in a beaker with the help of stirrer.
3. Transfer the contents into a volumetric flask. Add some more water (10-20ml) to the beaker and rinse the walls of beaker and add that to the same flask.
4. Raise the volume to 100 ml in the volumetric flask to prepare 5% NaCl solution.
5. Check the pH of the solution with pH meter.

Preparation of 5% hydrochloric acid solution (50ml) using 37% HCL.

Take 25 ml of distilled water in a volumetric flask.

Calculate the volume of HCL required by using the formula:

$C_1V_1=C_2V_2$

$37xV=5x50$

$V_1=250/37$

$=6.7$ ml

Add 6.7ml of HCl to the volumetric flask slowly and raise the volume to 50ml with distilled water to prepare 50 ml of 5%HCl solution.

Record the pH of the Solution using pH meter or litmus paper.

Experiment No. 2

Macroscopic analysis of Urine

Key recommendations for urine sample collection and handling:

- Patients should be well at baseline. They should have no urinary tract infection, no acute febrile illness, no intense exercise within the previous 24 hours.
- Recommended urine collection is a fresh, first morning void. A minimum of 5ml should be collected.
- If a first morning void is not practicable, random spot samples are acceptable.
- Samples not able to be delivered to the laboratory within 8 hours, should be refrigerated.
- Analysis should be performed on the day of receipt but samples can be stored for up to 7 days at 2-8 °C if necessary.
- Cloudy or particulate samples should be centrifuged prior to analysis.
- Positive ACR results must be confirmed, ideally on a fresh, first morning void, by repeat measurement on 1-2 occasions within 3 months.
- Prolonged storage should be at -70 °C; samples should not be stored at -20 °C.

Apparatus Required:

Plastic cup with a lid for sample collection, test tubes, test tube holder, dropper, beaker, litmus paper

Macroscopic Examination

The physical appearance of a urine sample can often tell a great deal about a patient's condition. A change in color or clarity may indicate the presence of a disease and the need for additional testing.

COLOR

Color is usually some shade of yellow and often varies with the concentration of the sample. The most common color descriptions of normal urine are straw, light yellow, yellow and dark yellow. Amber colored urine is seen in patients with increased bilirubin levels and may indicate hepatitis.

CLARITY

Clarity is an indication of the transparency of a specimen. It is best to judge clarity by observing light through a recently mixed sample. Terms used to describe clarity include clear, hazy, cloudy and turbid. Freshly voided urine that is properly collected is normally clear or slightly hazy, while contaminated urine is more likely to be hazy. Fresh urine that is cloudy is often the result of a

bacterial urinary tract infection due to white blood cells in the urine. Turbid urine contains salt crystals that precipitate out as the specimen cooled.

pH

The pH is a measure of the degree of acidity or alkalinity of the urine. A pH below 7 indicates acidic urine; pH above 7 indicates alkaline urine. Normal, freshly-voided urine may have a pH range of 5.5 - 8.0. The pH of urine may change with diet, medications, kidney disease, and metabolic diseases such as diabetes mellitus. Colors on the pH reagent pad usually range from yellow-orange for acidic pH to green-blue when pH is alkaline.

Experiment No. 3

Estimation of glucose levels in urine

Apparatus Required:

Plastic cup with a lid, test tubes, test tube holder, dropper, beaker, water bath

Theory:

A urine glucose test measures the level of glucose, or sugar, in urine. It is less invasive than a blood glucose test, but it also tends to be less accurate. High glucose levels often indicate diabetes, a group of diseases that affects the way the body handles glucose.

A urine glucose test is a quick and simple way to check for abnormally high levels of glucose in the urine. The most common cause of elevated glucose levels is diabetes, a condition that affects the ability to manage glucose levels. The symptoms of diabetes include excessive thirst, blurred vision, and fatigue. When left untreated, diabetes can lead to long-term complications, including kidney failure and nerve damage. Other diseases with elevated glucose levels can be renal glycosuria, diabetes, or gestational diabetes.

The normal amount of glucose in urine is 0 to 0.8 mmol/L (millimoles per liter).

Benedict's Test:

Benedict's test is used for the detection of reducing sugars(sugar shaving free reactive carbonyl group) in urine.

Principle:

Glucose is a simple aldehyde sugarwith the molecular formula C6H12O6.

Benedict's test utilizes a mixture of sodium citrate,copper(II) sulfate and sodium carbonate in a slightly basic solution. Reducing sugars reduce the copper(II)ionstocopper(I)oxide(Red ppt).

$$\textbf{R-CHO + 2Cu}^{2+}\textbf{+5OH}^{-} \longrightarrow \textbf{R-CO}_2^{-}\textbf{+Cu}_2\textbf{O (redppt) + 3H}_2\textbf{O}$$

R-CHO =Reducingcarbohydrates $R\text{-}CO^{2-}$ =Carbohydrate ion

Reagents Required

i. Benedict's Reagent**:** Dissolve Sodium Citrate(173g) and anhydrous Sodium Carbonate(100 g) in about 700ml of distilled water by gently heating the contents. Dissolve Copper Sulfate

(17.3g) in about 100mL of distilled water in a separate beaker.Transfer this solution gradually in to the Carbonate-Citrate mixture with constant stirring and raise the volume to1L with distilled water.

ii. Sample: Urine collected from diabetic and healthy subjects.

Procedure:

1. Add 1 mlof samplein a test tube.

2. Then add 2 mlof Benedict's reagent.

3. Heat thesolution in a boilingwaterbath for3minutes.

4. Formation of red precipitate indicates positive test.

Important notes:

Benedict's test is a semi quantitative test. The color of the precipitate gives a rough estimate of the reducing sugars present in the given sample.

Green color - Up to 0.5 g%

Green precipitate - 0.5-1.0 g%(+)

Yellow precipitate -1.0-1.5 g% (++)

Orange precipitate- 1.5-2.0 g% (+++)

Brick red precipitate- >2.0 g%

Fehling's Test

Apparatus Required:

Plastic cup with a lid, glass test tubes, holders, droppers, bottles for sample collection, test tube stand

Reagents Required

Fehling's solution A and B

Sample: Urine

Procedure:

In a test tube, add 2 ml of the test solution and add equal volumes of Fehling A & Fehling B and place it in a boiling water bath for few minutes.. When the contents of the test tube comes to boiling, mix them together and observe any change in color or precipitate formation. The production of yellow 'or brownish-red precipitate of cuprous oxide indicates the presence of reducing sugars in the given sample.

READINGS:

http://www.healthline.com/health/glucose-test-urine

Experiment No. 4

Estimation of albumin levels in urine

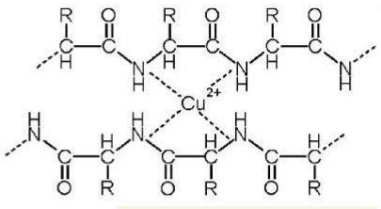
An albumin test checks urine for a protein called albumin. Albumin is normally found in the blood and filtered by the kidneys. If the amount of albumin is very small, but still abnormal, it is called microalbuminuria. Albuminuria is most often caused by kidney damage from diabetes (nephropathy) or chronic kidney disease. Screening for proteinuria is preferably done by measurement of urine albumin on a fresh, first morning void.

Biuret Test

This test is used forthe detection ofproteins containing at leasttwo peptidebonds.

Principle

In an alkaline medium, proteinreacts withcopper (Cu^{2+})in the Biuret reagent leading to the formation of violet colored complex. This increases the absorbance at 540nm due to that is directly proportional to the concentration of protein.



Biuret complex

Reagents Required

1. 10% sodium hydroxide solution
2. 1% copper sufate solution or Biuret reagent
3. Sample: Urine

Procedure

1. Add 1 ml of urine in a test tube.
2. Add 1 ml of 10% sodium hydroxide solution and 2-3 drops of 1% copper sulfate solution.
3. Mix well
4. Appearance of violet color will indicate presence of proteins in the sample.

Pre-analytical factors affecting urine albumin concentration

Patient	Sample
Hydration status	Sample clarity
Exercise	Collection type
Fever	Adsorption to plastic
Posture	Storage temperature

CALCULATION:

Total protein concentration (gm/ dL) = O.D. of Test X 5

OD of Standard

PRECAUTIONS:

1. Protect eyes fromsplash of the reagent.
2. Use automated pipettes and dispensing devices.

Heat and Acetic Acid Test (Protein)

Principle: based on precipitation by heat and coagulation by acids.

Procedure:

i. Fill test tube with urine (2/3 full) centrifuge.
ii. Heat the upper 2cm of the urine and observe the cloudiness. (Due to phosphates not albumin).
iii. Add 2 to 3 drops of 10% acetic acid .
iv. Cloudiness due to phosphates will disappear.
v. Repeat the heating. Persistent cloudiness indicates albumin. (Proteinuria)

Results:
If cloudiness developed at 40-60° C and disappears upon boiling but reappears on cooling, the protein present is called Bence-Jones protein. This protein is encountered in: Hyperglobulinemia - A condition characterized by abnormally large amounts of globulins in the blood. And in Multiple myeloma - also known as plasma cell myeloma, is the second-most common cancer of the blood.

Test For Bilirubin (Foam Test)

Principle: Bilirubin if present colors the foam yellow to green.

Procedure:

i. Place 5ml urine in a test tube. Place cover.
ii. Shake the urine vigorously for 3 mins.
iii. If Bilirubin is present, the foam produced will have a yellow to light green color.
iv. In patients with proteinuria, bilirubin bound to albumin can also appear in urine.

READINGS:

- http://www.healthline.com/health/glucose-test-urine

- Martin H., 2011. Laboratory Measurement of Urine Albumin and Urine Total Protein in Screening for Proteinuria in Chronic Kidney Disease.Clin.Biochem. Rev. Vol 32

Experiment No. 5

Estimation of chloride and phosphate levels in urine

1. **For Chlorides (Fantus Test)**

Principle:

$AgNO_3$ reacts with the chloride in urine to precipitate AgCl. Any excess $AgNO_3$ reacts with Potassium Chromate to form reddish ppt. of Ag_2CrO_4 (Silver chromate). The appearance of which indicates end point of reaction.

Procedure:

i. Place 10 drops of urine to a test tube and one drop CrO_4 solution as indicator.
ii. Add drop by drop 2.9% $AgNO_3$ solution until a permanent red brown color (end point) is developed.
iii. Number of drops consumed represent amount of chloride present. Normally 6 to12 drops.
iv. May indicate Hyperchloremia if it exceed 12.

2. **Test for Urinary phosphates Ammonium molybdate test (Test for Urinary phosphates)**

Principle:

Upon warming with ammonium molybdate in the presence of nitric acid, inorganic phosphates are precipitated as canary yellow ammonium phosphomolybdate.

Procedure:

i. To 3 ml of urine, add a few drops of concentrated nitric acid and a pinch of ammonium molybdate.
ii. Warm it.
iii. Observe the yellow color of the precipitate.

Test for Urinary phosphates Interpretation:

a) Increased urinary phosphates: Rickets, osteomalacia, hyperparathyroidism, acidosis.

b) Decreased urinary phosphates: Diarrhea, nephritis, parathyroid hypofunction, pregnancy, hereditary fructose intolerance and galactosemia.

Experiment No. 6

Kidney function tests (RFTs)

AIM: To estimate level of Blood SGOT (serum glutamic-oxaloacetic transaminase) (Liver function test)

REAGENTUSED:

Sample: Venous Blood in plain bulb: (0.05 mL Serumis required)

Reagents:

Reagent1: Buffered Aspartate –KG Substrate, pH7.4

Reagent2: DNPH Color Reagent (Dinitrophenylhydrazine)

Reagent3: SodiumHydroxide, 4N

Reagent4: Working Pyruvate Standard, 2mM

Preparation of Working Solutions

Solution1: Dilute 1 mLofReagent 3 to10mL withPurified Water. Reagent 1, 2 and 4 are ready –to- use.

APPARATUS USED:

Test tubes, Test tubestand, Pipettes, Micropipettes, Spectrophotometer

CLINICAL SIGNIFICANCE:

Elevated levels of GOTs are found in Myocardial Infarction.The duration and extent of increase is related to the size of the Infarct. In the differentiation of Myocardial Infarction from other Cardiac disorders,GOT determination is of considerable value.GOT levels are also elevated in various types of Liver diseases,in Skeletal Muscle Traumaand sometimes in renal diseases.

PRINCIPLE:

GOT (AST) (Aspartate aminotransferase) catalyses the following reaction:

Ketoglutarate + L-Aspartate ⟶ L-Glutamate + Oxaloacetate

Oxaloacetates of ormedis coupled with2,4-Dinitrophenylhydrazine(2,4-DNPH)to give the corresponding hydrazone, which gives Brown color in alkaline mediumand this can be measured calorimetrically.

PROCEDURE:

Take 0.25mL reagent 1 in a test tube and incubate at 37ºC for 5 minutes. Now add 0.05mL serum and mix it well and again in cubate it 37ºC for 60 minutes. Add 2.5mL of solution 1 in this mixture (also given in table).

Pipette into tube marked	Test (T)
Reagent 1	0.25 ML
Incubate at 37 ºC for 5 minutes	
Serum	0.05mL
Mix well and incubate at 37ºC for 60 minutes	
Solution 1	2.5 mL

Mix well and allow to stand at Room Temperature (15 -30 ºC) for 10 minutes and take the reading (OD) aginstpurified water on a colorimeter using a Green filter or on Photometer at 505 nm.

PROCEDURE FOR STANDARD CURVE

As the reaction proceeds with time, more amounts of products are formed. Since the end products inhibit the enzyme, there is more of Inhibition. This is the major problem with colorimetric methods for the estimation of this enzyme.

On the other hand In Kinetic methods,since the enzyme activity is measured during the Initial few minutes, the amount of products formed during that short time are negligible to cause any Inhibition.Because of the above problem,it is necessary to standardize any colorimetric method against a Standard kinetic method. So this standardization is done against the Standard Karmen Unit Assay (Kinetic) and this is extrapolated to different amounts of Pyruvateandthishas been thoroughly rechecked. At this point it is important to note that the Standard graph of Enzyme activity (IU)on x-axis vs. OD on Y-axis is not a linear one, which shows that O.D. increases with increases in enzyme activity at a decreasing rate.

It Is not necessary to plot Standard curve every time at est is performed. It should be plotted initially when the first test is performed.

Subsequently, periodic checking can be done by running only a couple of tubes viz.tubes,1 and 3 of the following table and their OD can be compared with the original curve. Each laboratory should establish its own standard curve as per the below mentioned procedure table.

Tube No.	1	2	3	4	5
Assigned Enzyme activity (IU/L)	0	24	61	114	190
Reagent to be pipette	Volume in mL				
Reagent 1	0.5	0.45	0.4	0.35	0.3
Reagent 4	-	0.05	0.1	0.15	0.2
Purified Water	0.1	0.1	0.1	0.1	0.1
Reagent 2	0.5	0.5	0.5	0.5	0.5
Mix well and allow to stand at Room Temperature (15 - 30· C) for 20					
Solution I	5.0	5.0	5.0	5.0	5.0

Mix well by inversion. Allow to standat Room Temperature (15-30ºC) for 10minutes and measure the O.D of all the five tubes against purified water on a colorimeter with a green filteroron photometer at 505 nm. Plot a standard graph by taking enzymeactivity on X-axis and O.D. on Y-axis.

OBSERVATION TABLE:

S. No.	Reading for standard	Optical density (OD) at 505 nm
1.	1	
2.	2	
3.	3	
4.	4	
5.	5	

S. No.	Reading for tubes	Optical density (OD) at 505 nm
1.	Control	
2.	Test	

CALCULATIONS:

Mark the reading (OD) of Test (T) on the Y axis of the Standard curve and extrapolate it to the corresponding enzyme activity on X-axis.

PRECAUTIONS:

1. Serum samples must be completely free from haemolysis,since RBCs are very rich in this enzyme.
2. Haemolysis leads to the release of large amount of the enzyme andhence gives erroneous results.
3. Avoid the use of detergents to clean glassware.
4. Use clean and dry glassware.
5. Reagent 3 is corrosive; avoid contact with skin

Experiment No. 7

Liver function tests (LFTs)

AIM: To estimate level of Blood SGPT (Liver function test)

REAGENTUSED:

Sample: Venous Blood in plain bulb: (0.05 mL Serumis required)

Reagents: Reagent 1: Buffered Alanine -- KG Substrate (pH7.4) Reagent 2: DNPH colour Reagent
Reagent 3: SodiumHydroxide, 4N
Reagent 4: Working Pyruvate Standard, 2mM

Preparation of working solutions:

Solution 1: Dilute 1 mLofReagent 3 to 10 mL with Purified Water. Reagent 1, 2 and 4 are ready - to - use.

APPARATUS USED:

Test tubes, Test tubestand, Pipettes, Micropipettes, Spectrophotometer

CLINICALSIGNIFICANCE:

ElevatedlevelsofGPTsarefoundinliverdiseases.ValueofSGPTisverymuchhigherthanthenormalin case of infective hepatitis and the rise begin with the prodermal period, helping early diagnosis.

PRINCIPLE:

Analysis of SGPT is carried out using 2, 4-DNPH as colouring reagent.
SGPT (ALT) Catalyses the followingreaction:

α-Ketoglutarate + L-Alanine ⟶ L-Glutamate + Pyruvate

Pyruvates of ormedis coupled with2,4-Dinitrophenylhydrazine(2,4-DNPH)to give the corresponding hydrazone, which gives Brown color in alkaline mediumand this can be measured colorimetrically.

PROCEDURE:

Take 0.25mL reagent1 in a test tube and incubate at 37ºC for 5minutes. Now add 0.05mL serum and mix it well and again incubate it 37ºC for 60 minutes.Add 2.5mL of solution 1 in this mixture (also given intable).

Pipette into tube marked	Test (T)
Reagent 1	0.25 ML
Incubate at 37 ºC for 5 minutes	
Serum	0.05mL
Mix well and incubate at 37ºC for 60 minutes	
Solution 1	2.5 mL

Mix well and allow to stand at Room Temperature (15 -30 ºC) for 10 minutes and take the reading (OD) aginstpurified water on a colorimeter using a Green filter or on Photometer at 505 nm.

PROCEDURE FOR STANDARD CURVE

As the reaction proceeds with time,more amounts of products are formed.Since the end products inhibit the enzyme,there is more of Inhibition.This I sthe major problem with colorimetric methods for the estimation of this enzyme.On the other hand In Kinetic methods,since the enzyme activity is measured during the Initial few minutes, the amount of products formed during that short time are negligible to cause any Inhibition. Because of the above problem, it is necessary to standardize any colorimetric method against a Standard kinetic method. So this standardization is done against the Standard Karmen Unit Assay (Kinetic) and this is extrapolated to different amounts of Pyruvate and this has been thoroughly rechecked. At this point it is important to not ethat the Standard graph of Enzyme activity (lU) on x-axis vs. Odon Y-axis is notalinearone,which shows that O.D.increases with increases in enzyme activity at a decreasing rate.A Sample Curve is given in Fig.
It is not necessary to plot Standard curve every time at estis performed.It should be plotted initially when the first test is performed.
Subsequently, periodic checking can be done by running only a couple of tubes viz.tubes,1 and 3 of the following table and their OD can be compared with the original curve. Each laboratory should establish its own standard curve as per the below mentioned procedure table.

Tube No.	1	2	3	4	5
Assigned Enzyme activity (IU/L)	0	24	61	114	190
Reagent to be pipette	Volume in mL				
Reagent 1	0.5	0.45	0.4	0.35	0.3
Reagent 4	-	0.05	0.1	0.15	0.2
Purified Water	0.1	0.1	0.1	0.1	0.1
Reagent 2	0.5	0.5	0.5	0.5	0.5
Mix well and allow to stand at Room Temperature (15 - 30· C) for 20					
Solution I	5.0	5.0	5.0	5.0	5. 5.0

Mix well by inversion. Allow to stand at Room Temperature (15-30ºC) for 10 minutes and measure the O.Do fall the five tubes against purified water on a colorimeter with a green filteror on photometer at 505 nm. Plot a standard graph by taking enzyme activity on X-axis and O.D. on Y-axis.

OBSERVATION TABLE:

S. No.	Reading for standard	Optical density (OD) at 505 nm
1.	1	
2.	2	
3.	3	
4.	4	
5.	5	

S. No.	Reading for tubes	Optical density (OD) at 505 nm
1.	Control	
2.	Test	

CALCULATIONS:

Mark the reading (OD) of Test (T) on the Y axis of the Standard curve and extrapolate it to the corresponding enzyme activity on X-axis.

PRECAUTIONS:

1) Serum Samples must be completely free from haemolysis, since RBCs are very rich in this enzyme.

Haemolysis leads to the release of large amount of the enzyme and hence gives erroneous results.

2) Avoid the use of detergents to clean glassware.

3) Use clean and dry glassware.

4) Reagent 3 is corrosive; avoid contact with skin.

NOTES:

1)Serum samples can be store dat2-8·C (Refrigerator) for 5 days without any substantial loss of the enzyme activity.

2) A separate Blank is not necessary, since tube No.1 ofStandard curve substitutes the Blank

3) If the enzyme activity is more than 190 units,repeat the Test using the Serum diluted1:5(or more if necessary) with Normal Saline and multiply the final result so obtained with an appropriate factor.

NORMAL VALUES:

- The normal range of values for AST (SGOT) is from 5 to 40 units per liter of serum (5-40 IU/L)
- The normal range of values for ALT (SGPT) is from 7 to 56 units per liter of serum.

Experiment No. 8

Study of abnormal urine content bile pigment and salts.

REAGENT USED:

Sample: Urine

Reagents:

Nitricacid (HNO_3), Silvernitrate ($AgNO_3$), Sodiumhydroxide(NaOH), Aceticacid(CH_3COOH), $BaCl_2$, Sulphur particles

APPARATUS USED:

Test tubes, Test tube stand,Pipettes, Measuring cylinder.

CLINICALSIGNIFICANCE:

Abnormal urine conditions shows dysfunctionin kidney's filtrationprocess that may occur due to various disease conditions. Mainly presence of bile pigments and biles altsin urine samples are in dication of jaundice.

PROCEDURE:

A)Test of Inorganic constituents:

i) **Test for chloride ion:**

Took 1ml of urine and added 3 drops of diluted HNO_3 and 4 drops of dilute $AgNO_3$.Appearance of white precipitate indicate chloride ion.

ii) **Testforphosphateion:** Took 1ml of urine and added 4drops of dilute NaOH. Heated and appearance of white precipitate indicate the presence of phosphate ion.

iii) **Test forsulphateion:** Took 1ml of urine and added 3 drops of dilute CH_3COOH and 4drops of $BaCl_2$. Appearance of white precipitate indicate sulphate ion.

B) Test for organic constituents:

Test for bile Pigment: Took 5ml of fuming HNO_3in test tube and added 3ml of urine from side of tube. Appearance of green or blue of reddish yellow colour indicate the presence of bile pigment in the urine.

ii) **Test for Bile Salts:** To 1ml of urine added sulphur particles,if sulphur immerse in urine than Bile salts are present and if it floats then bile salts are absent.

OBSERVATION TABLE:

S. No.	EXPERIMENT	OBSERVATION	INFERENCE
1			
2			
3			

4			
5			

PRECAUTIONS:

1. Use clean and dry glassware.
2. Avoid regents to be splashed in eyes.