# URINE

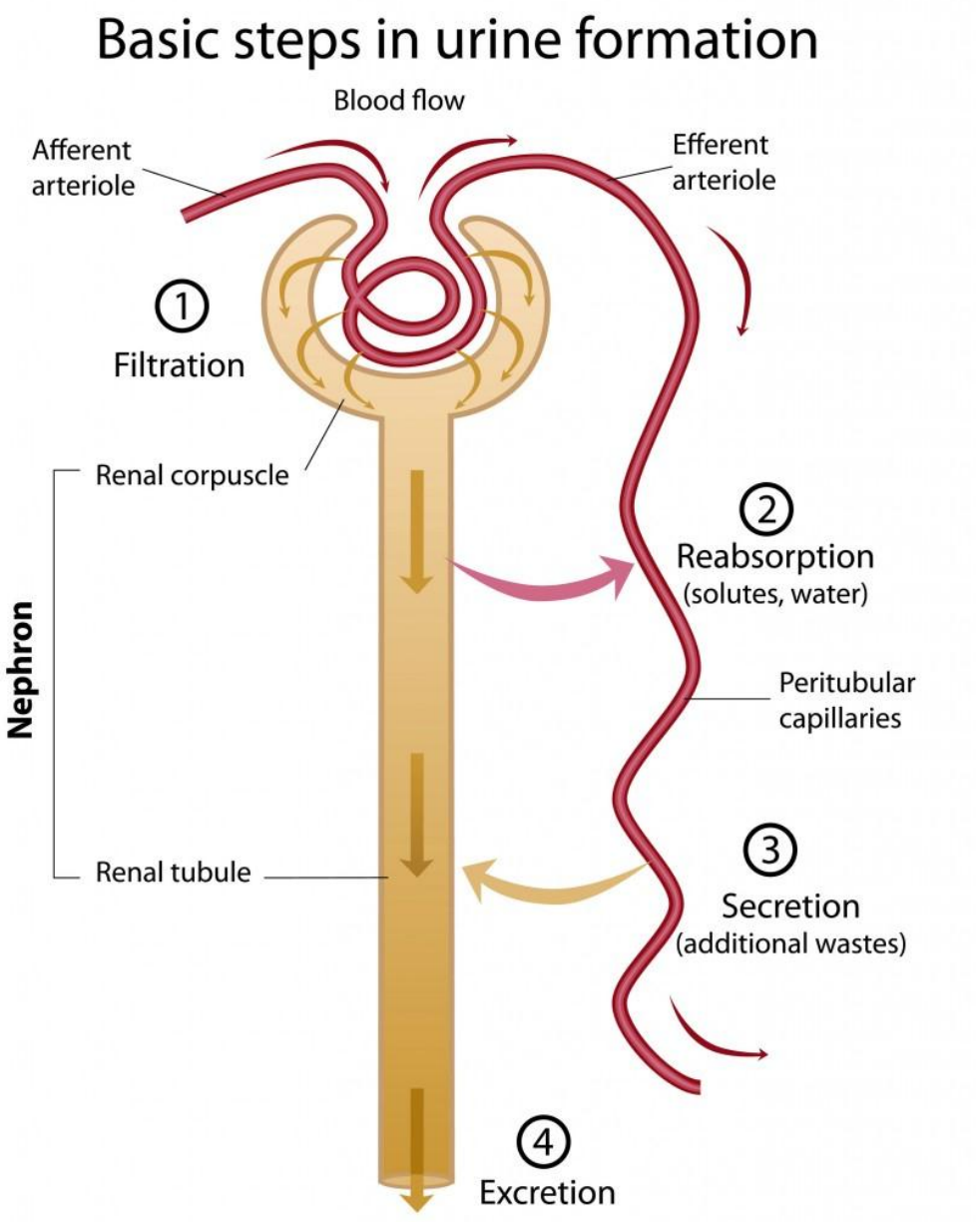
## INTRODUCTION:

Urine is one of the biological fluids that is responsible for the removal of toxic substances from the body. It was pale yellow coloured fluid an average sample of urine has about 95-96% water, 4% solids dissolved in it of these approximately 2% is urea, and all other organic and inorganic constituents make up the remaining 2-3% urea, uric acid, creatinine and ammonium salts, vitamin C, oxalic acid and phenolic substances are the principle nitrogenous compound. Daily urine output is 1.5 to 1.8 it's the volume of urine output proportional to the fluid intake.

The urinary system consists ot two kidneys, two ureters, one bladder and one urethra. The formation of urine for the production of urine, the kidneys do not simply pick waste products out of the bloodstream and send them along for final disposal. The kidney's 2 million or more nephrons (about a million in each kidney) from urine by four precisely regulated processes:

1. **Glomular filtration:** Movement of materials under pressure from the blood into the capsule through glomerular membrane is therefore known glomerular filtrate the blood cells and large protein molecules cannot pass.
2. **Tubular reabsorption:** Movements useful substances ( water, nutrients and ions) back into the blood while keeping waste products in the nephron to be eliminated in the urine.
3. **Tubular secretion:** Movement additional substances from the blood into the nephron for elimination. Movement of hydrogen ions is one mean is one means by which the pH of body fluids is balanced.

4. **Concentration of the urine:** The amounts of water that is eliminated with the urine is regulated by a complex mechanism within the nephron that is influenced by antidiuretic hormone (ADH), a hormone released from the posterior pituitary gland.




## Collection of urine specimens

Single specimen of urine is used for general urine examination and for most qualitative testes. For quantitative work, 24 hour specimen is best employed, except when collecting specimens as part of tests such as D-xylose test.

## Kinds of samples

### 1. Random sample

In which the concentration of its component is different from sample to other due to physiological state, kind of diet, fluid intake.

### 2. Morning sample:

Used for pregnancy test and general urine examination GUT. Its component is in highest conc.

### 3. Limited time sample.

### 4. Bacteriological test sample.

### 5. Collection of urine from chidren

Collection of a timed specimen from a baby is difficult, but fortunally such specimens are rarely required. The scrotal or perineal area is first cleaned and dried, and any natural or applied skin oils are removed. For an untimed specimen, a plastic bag (U-bag, Hollister Inc., Chicago, IL or Tink-Col, C.R.Bard Inc., Murray Hill, NJ) is placed around the infant's genitalia and left in place until urine has been voided.

### 6. 24 hour sample

For collecting a 24 sample, the patient empties the bladder first and the urine is discarded. All specimens passed therefore during the day and during the following night are saved and the specimen obtained by emptying the bladder at the same time the following morning is added to them.

The sample is collected in clean covered container and kept in a cool place preferably in the refrigerator. If urine has to be kept, it may be necessary to prevent the effect of bacteria by adding a proper preservative.

**Urine preservatives**

Urine may be preserved for quantitative examination in a refrigerator (avoiding freezing) or a small pieces of camphor or thymol added other preservative are formalin and toluene 2% for micros one to two drops of formalin (for 30 ml of urine can be used as preservative).

1. **Hydrochloric acid:**

   Hydrochloric acid is most commonly used as presertative, 10 ml of concentrated hydrochloric acid or 100 ml of 1N (diluted HCl) it is enough for 24 hr sample it is suitable in case of nitrogen, ammonia, calcium and phosphate estimation.

   Ureate is precipitate with HCl so it is unsuitable preserviaent other acids like $H_2SO_4$ may be used 1 ml of the acid is enough for 24 hr. sample is suitable determination of Gatecolamine.

2. **Toluene**

   This is used as preservative for 24 hr sample in case of determination of sodium, potassium, uric acid and proteins (10) ml of it is enough.

3. **Glacial acetic acid**

   (10) ml of it is used as presertative for 24 hr sample in case of determination of ascorbic acid and 5-hydroxy indole glacial acetic acid.

4. **Hibitane**

   Small amount of (S%) hibitane used as preservative in case of determination of glucose. Also chloroform, formalin, petroleum ether, boric acid and some others can be used. The preservative used must be identified on the table of the container. If no preservative is used, this too should be noted.

# URINALYSIS

## General urine examination GUE

Urine examination should include:

**I. Physical examination**

1. Color.
2. Odor.
3. Appearance.
4. Reaction (pH).
5. Specific gravity.

**II. Biochemical examination**

1. Glucose.
2. Protein
3. Ketone bodies
4. Bilirubin and urobilinogen

**III. Examination of urinary deposit**

**Physical examination**

**1. Color.**

Urochrome and uroerythrin are pigments which give normal its characteristic colour (light yellow). Small quantities of blood give urine a smoky appearance while larger quantities make it brownish or red.

Bile pigments cause the urine to appear brown with the production of yellow froth when the urine is shaken in a test tube. Drugs may also lead to discolorations of urine.

**2. Odour**

Normal urine has aromatic and on standing it is ammoniac due to bacterial activity. Some disease cause a change in the odour of urine.

Fruity or sweetish – diabetes or starvation (Keton bodies).

Putride – $H_2S$ liberation. In pus of cystitis, cystinuria.

Faccal – due to contamination with faces or coliform ammonical – due to bacterial action.

3. **Appearance.**

   Normal urine is quite transparent when freshly passed but it may be opalescent from the presence of various substance in suspension of which the most importance are pus bacteria and phosphates. Record the colour and appearance of your sample.

4. **Reaction**

   Normal urine is slightly acidic with pH range between (6-7) with the help of pH paper determine and record the approximate pH of the urine provided.

5. **Specific gravity:**

   Specific gravity ( Sp.G ): is the term that used to comparison between the weight of identified volume of liquid with the weight of the same volume of water.

**Procedure:**

1. Fill a suitable sized cylinder with the urine (Shaking it before used).
2. Place a hydrometer in the fluids taking care that it floats and does not touch the sides of the cylinder. Read the sp.Gr.

   The normal average range of specific gravity of urine varies from 1.010-1.025.

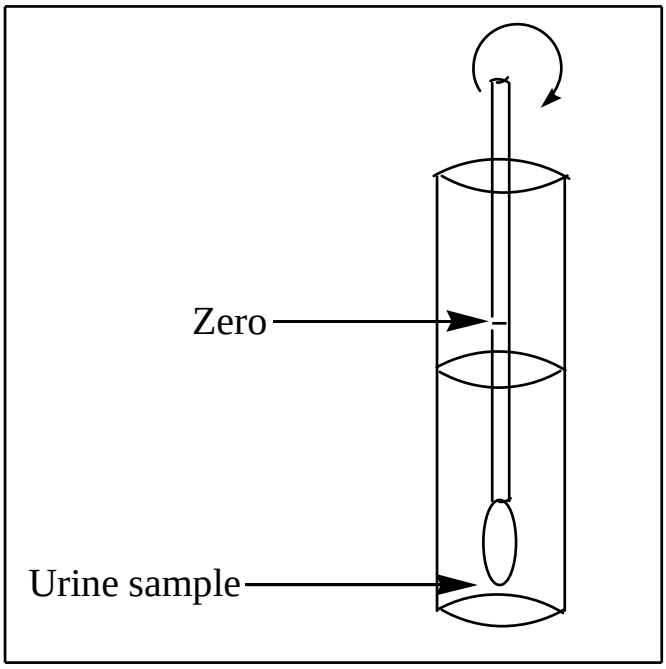



**Fig.2: specific gravity**

The zero of a hydrometer equivalent (1000). More than (1000) indicate the presence of solid constituent so we can measure the amount of the total solids from the specific gravity.

Specific gravity of urine increase in case of dehydration, fevers, heavy proteinuria, glucosuria and acute kidney function. While, it decreased in case of diabetes insipidus, nephritic syndrum and diuretic use.

**Example:** the Sp.Gr. of a urine sample is 1012 find the total solids of this sample?

(sp.Gr. of urine-sp.Gr. of water)× constant factor (2.6) = (g/L) solid oupoud

1012-1000 = 12

Total solid (T.S.) = 12×2.6 g/L

We must observed the temperature of the sample because the hydrometer is degresed at 15°C so 0.001 must be added for each 3°C more than 15°C. (correction should be made).

**Example:** the Sp.Gr. of a urine sample was 1015 at 24°C so is corrected as follow:

$$\frac{\textbf{Lab.temp} - \textbf{15}}{\textbf{3}}$$

$$\frac{24-15}{3} = \frac{9}{3} = 3$$

The correct sp.gr. is

1015 +3 = 1018

$$\text{If the Lab. temp. is } 9^{\circ}\text{C so the sp.gr.} \quad \frac{15-9}{3} = \frac{6}{3} = 2$$

= correct sp.gr. = 1015 -2 = 1013

The case of only little amount of urine sample available the sp.gr. can be found by comparism weight of specific volume of urine to the same volume of water.

**Example:** the weight of 1 ml of urine sample at 25°C is (1.016) and weight of 1 ml of distill water at the same temp. Is (0.996). calculate the sp.gr. of this sample?

$$\text{sp. Gr.} = \frac{1.016}{0.996} = 1.020$$

## Normal inorganic constituents of urine

Among the inorganic substances present are:

chloride ($Cl^-$)

phosphate ($PO_4^{-3}$)

sulphate ($SO_4^{=}$)

bicarbonate ($HCO_3^-$)

Sodium ($Na^+$), potassium ($K^+$), calcium ($Ca^{++}$), magnisum ($Mg^+$) and ammonium ($NH_4^+$).

### 1. Chloride $Cl^-$

The chloride excreted in urine as sodium chloride (NaCl). The amount of NaCl excreted is about (10-15) g/day and it is decreased in case of vomiting and sweating.

**Method of determination:**

**Materials**

1. Urine sample.
2. Conc. $HNO_3$.
3. $AgNO_3$ (5%).

**Procedure:**

1. Take (1) ml of urine in a test tube.
2. Add drop of conc. $HNO_3$.
3. Add (2) drops of $AgNO_3$ sol.

White precipitate indicate the presence of $Cl^-$.

## 2. Sulphates $SO_4^{=}$

It is derived from the metabolism of amino acids which contains the sulphur ( cystein, cystine, methionine…) .

**Materials:**

1. Urine.
2. Conc. HCl.
3. $BaCl_2$ sol (100g/L).

**Procedure:**

1. Take 1 ml of urine.
2. Add drop of conc. HCl.
3. two drops of $BaCl_2$ solution.

   Amilkiness precipitate indicates the presence of sulfate in urine.

## 3. phosphate $PO_4^{\equiv}$

One of the importance sources of phosphate is the metabolism of amino acids, fatty acids, phosphoprotein and the phosphosaccarides carbohydrate.

**Material:**

1. urine sample.
2. Conc. $HNO_3$.
3. Ammonium molybdate solution. (10%).

**Procedure:**

1. Take 1 ml of urine.
2. Add 3 drops of conc. $HNO_3$.

3. Add 1 ml of ammonium molybdate solution.
4. Heat and allow to stand in the rack.
5. A yellow-green precipitate of ammonium phosphomolybdate indicate the presence of phosphate.

**4. Ammonium salt $NH_4^+$**

The ammonium salt is generated from glutamine as below.

$$\text{Glutamine} \xrightarrow{\text{Glutaminase}} \text{Glutamate} + NH_4^+$$

$$NH_4^+ + OH^- \xrightarrow{\text{Decomposition}} NH_3\uparrow + H_2O$$

**Materials:**

1. Urine sample.
2. Red litmus paper.

**Procedure:**

Take 2 ml of urine, boil for 3 min. Then put a moist red litmus paper at the neck of the test tube, ammonia which liberated due to the effect of boiling will change the colour of litmus paper to blue.

Moreover, we can used phenophthaline (ph.ph.) as indicator to presence of ammonia, that the pink color of ph.ph. in alkaline medium converted to colorless in acidic medium.

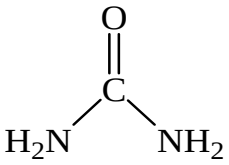
## Nitrogenous substances in urine

Nitrogenous substances include

1. Urea.
2. Uric acid.
3. Creatinine.
4. Ammonium salts.
5. Indican ($C_{14}H_{17}NO_8$).

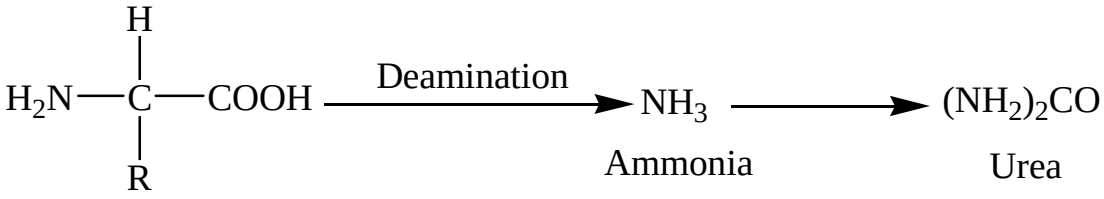
## 1. Urea

**Chemical formula**



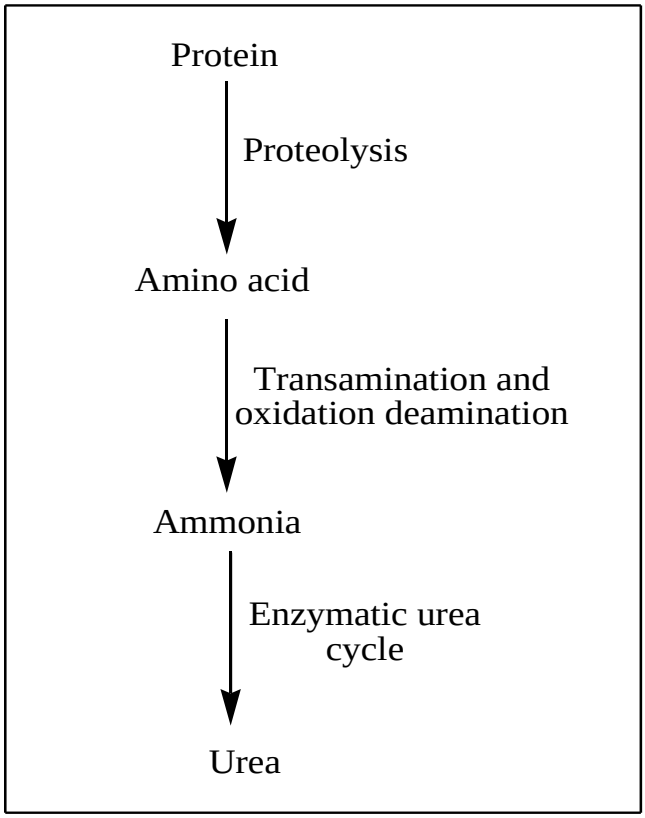

**Biosynthesis of urea:**

Urea is the major end product of amino acid catabolism, it is synthesized in the liver.




Amino acid

The biosynthesis of urea from ammonia is carried out exclusively by hepatic enzymes of the urea cycle.




Over 90% of urea is excreted through the kidney. It is filtered freely by the glomerulus and neither activity reabsorbed nor secreted by the tubules. However passive tubular reabsorption occurs to a significant extent.

Although serum urea concentration is often used as an index of renal glomerular function, measurement of serum creatinine provides a more accurate assessment.

Urea production is increased by a high protein intake, in catabolic state and dehydration., conversely, production of urea is decreased in patients with low protein intake and sometimes in patient with liver disease. Also blood urea nitrogen (BUN) may be reported. Since the molecular weight of urea is boglmol and the molecular weight of nitrogen in 14/mol so 60 g of urea contain 28 g of nitrogen. BUN can be calculated as follow:

$$BUN = \frac{\text{Urea amount}}{2.16}$$

The normal range of serum is 3.3 - 6.6 mmol (20-40 mg/dL) and the amount excreted is about 2%.

**Materials:**

1. Urine.
2. Sodium hypobromite.
3. (1%) urea.
4. (10%) sodium hydroxide.
5. (0.5%) cupper sulphate.

**Procedure (1):**

Prepare and label two test tubes:

- first tube: 2 ml of urine.
- second tube: 2 ml of 1% urea solution.

Add to each tube 10 drops of sodium hypobromite, effervescence indicates the presence of urea:

$$(NH_2)_2CO + 3\,NaOBr \longrightarrow NaBr + H_2 + CO_2 + 2\,H_2O$$

**Procedure (2):**

This procedure requires isolation of urea from urine by some methods and this take along time, so instead of urine we used solid urea (small quantities) put it in dry, clean and heat resistance test tube and then we put red litmus paper on the neck of the test tube, then heat directly, changes the colour of litmus paper to blue indicate the libration of ammonia. (which related with urea condensation) after that used biuret test on the ppt, on the botton of the test tube precipitate by adding

1. (1) ml of NaOH solution.

2. (2) drops of CuSO4 solution.

Violet colour indicate the presence of peptide bond (condence urea). Repeat biuret test on solid urea, urine, (1%) of urea solution. Compare and record your results.

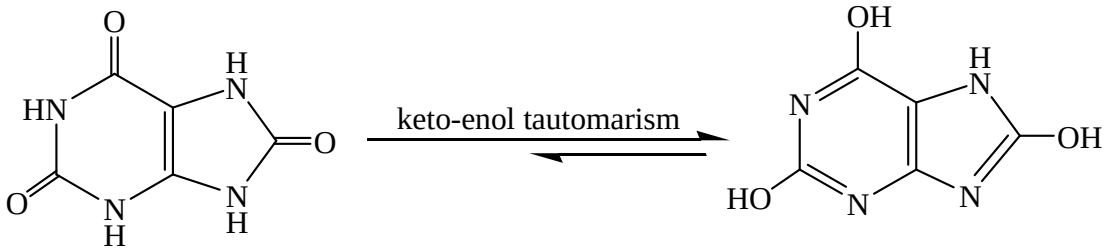
Solid urea + 1 ml NaOH + drop by drop $CuSO_4$ $\xrightarrow{\text{Mix.}}$ +ve (violet colour)

(1) ml urine + (1) ml NaOH + drop by drop $CuSO_4$ $\xrightarrow{\text{Mix.}}$ -ve (blue colour)

(1) ml urea sol. + (1) ml NaOH + drop by drop $CuSO_4$ $\xrightarrow{\text{Mix.}}$ -ve (blue colour)

## 2.Uric acid

Chemical formula




Keto form

Enol form

**Classification**

It is a form of non protein nitrogenous compound (NPN).

**Biosynthesis of uric acid:**

Uric acid is the end product of purine metabolism. It is a waste product derived from purins of the diet and those synthesized in the body. In human, uric acid arises from ingested nucleoproteins, degradation of nucleoproteins in nuclear material and by synthesis from simple precursors.

Healthy adult human body contains about 1.1 g of uric acid. Normally about on half of uric acid is eliminated and replaced each day, partly by urinary excretion and partly through destruction in the intestinal tract by microorganisims.

Serum uric acid is freely filtered by the glomeruli and (98-100%) of it is subsequent reabsorbed in the proximal tubles which is then followed by further secretion in the tubules. The normal of levels of uric acid in urine is (250-750 mg/day).

**1.Follin's test:**

**Materials:**

1. Urine.
2. 0.1 % uric acid.
3. Sodium carbonate 10%.
4. Folin reagent (Phosphotungstic acid reagent 5%).

**Procedure:**

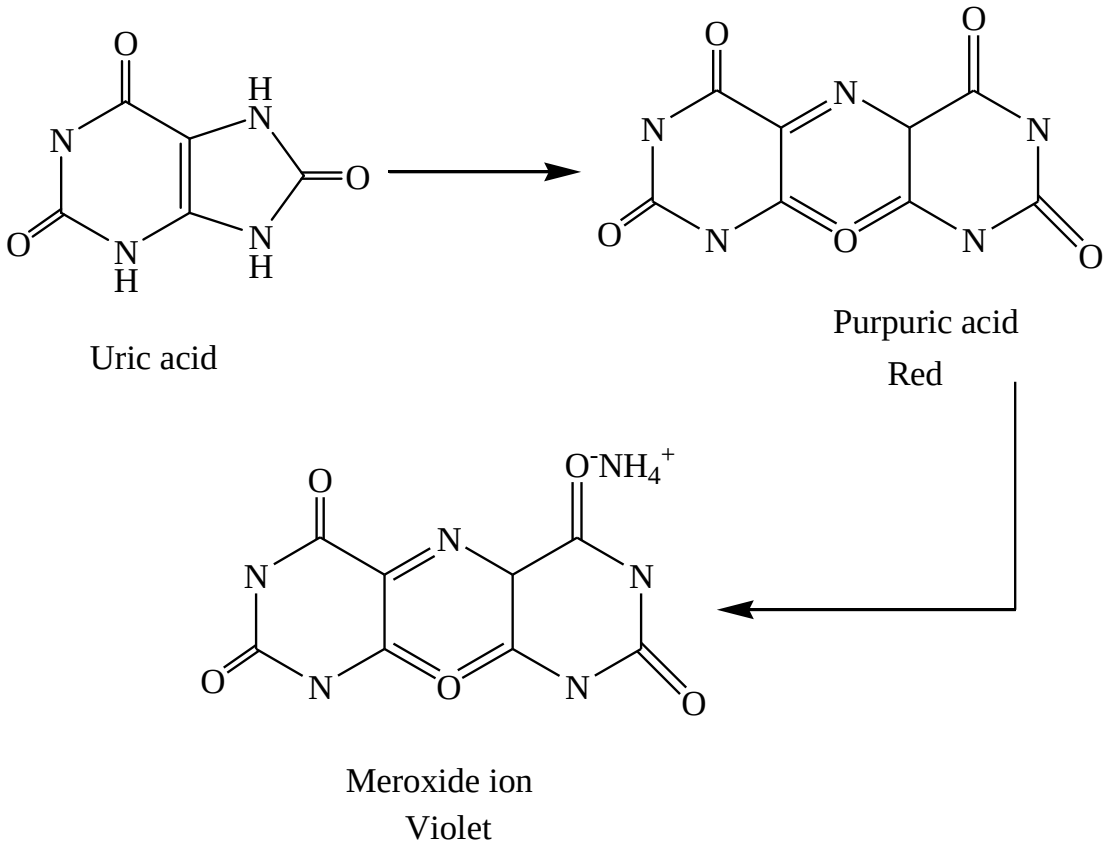
Prepare and label two test tubes

1. (2) ml of urine, the other 2 ml of 1% uric acid.
2. Add (2.3) drops of (Folin reagent), Mix.
3. Add (3-5) drops of 10% $Na_2CO_3$.
4. Mix, deep blue colour.

**2. Meroxide test for uric acid:**

This method need isolated uric acid from urine and this takes long time therefore we used solid uric acid.

**Principle:**

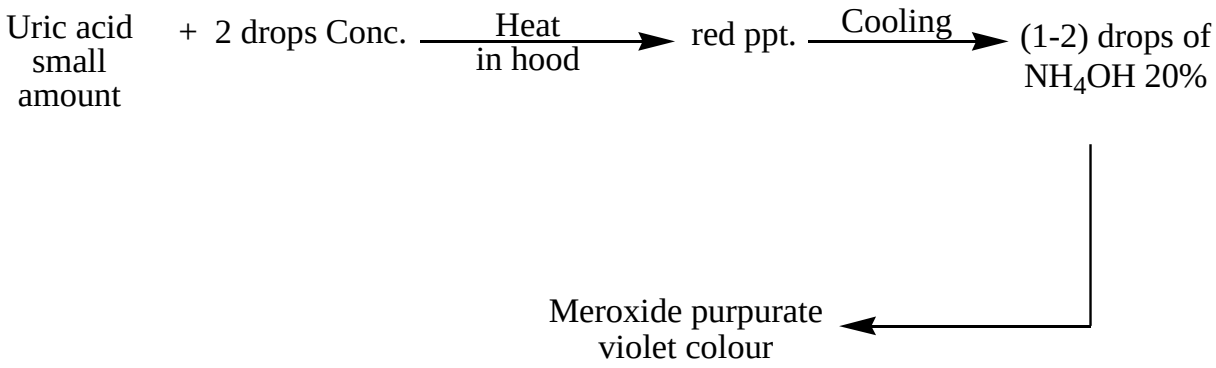



**Materials:**

1. Uric acid (solid).
2. Conc. nitric acid ($HNO_3$).
3. Ammonium solution (20% $NH_4OH$).

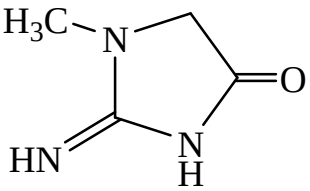
**Procedure:**

1. Put small amount of solid uric acid (We can separated it from urine by some method).
2. Add 2 drops of conc. nitric acid ($HNO_3$).
3. Heat directly in hood, red precipitate of purpuric acid is formed.
4. Cool and add (1-2) drops of ammonium hydroxide, violet colour indicate the present of uric acid.




## 3.Creatinine

**Chemical formula:**




Creatinine

## Classification:

Each of creatine and creatinine is non protein nitrogenous compound (NPN) which present in low concentration in healthy human blood.

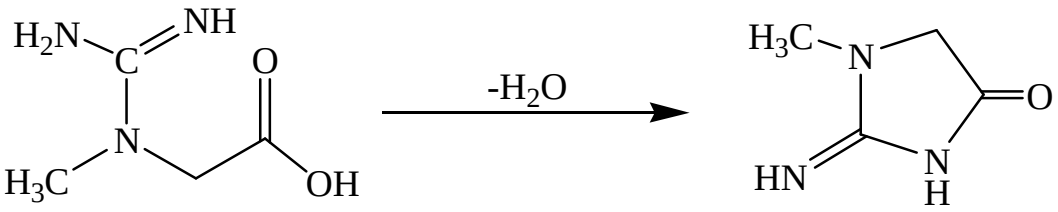
## Biosynthesis:

Creatine is synthesized in the kidney, liver and pancrease. Creatine is the then transported to body organs such as muscle and brain where it is phosphorylated to phosphocreatine, both creatine and phosphocreatine change to creatinine to about 2% in a day.

Creatinine is extracted mainly by the kidney following filtration, no further reabsorption of creatinine will occur through the tubules, small quantity of creatinine is secreted in the tubules (7-10%).

As a result, creatinine clearance represent an indicator of glomerular filtration rate (GRF). Creatinine has a constant range. It is measurement is used to evaluate (particularly glomerular) function. Urine creatinine (24 hr. sample) values can range from 500 to 2000 mg/day. Results depend greatly on age and amount of leanbody mas.

## Jaff's test for creatinine:




Creatine Creatinine

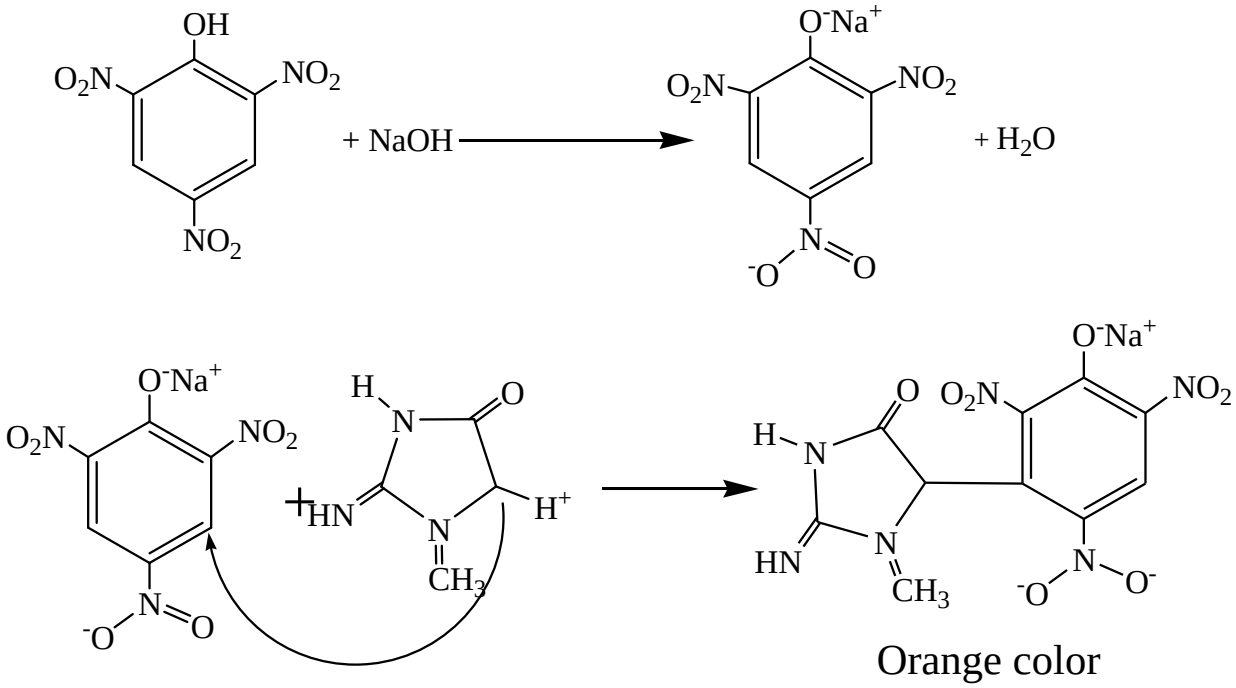
**Material:**

1. Saturated picric acid.
2. 10% NaOH.
3. Urine sample.
4. Distill water (D.W).

**Procedure:**

1. To 1 ml of saturated picric acid add 0.5 ml of 10% NaOH.
2. Divide equally into test tubes.
3. To one of the test tubes add 1 ml of urine and to the other add an equal volume of water. A deep redish-orange indicates the presence of creatinine due to the formation of creatinine picrate.

   Principle:




## Abnormal constituents of urine

Substances which are not present in easily detectable quantities in the urine of healthy person but occur in urine under certain disease conditions are called abnormal constituent urine. The detection of these substances in urine is a matter of great clinical importance.

### 1. Proteins:

The excretion of easily detectable amounts of coagulable protein in urine is termed "Albuminuria" Albumin and varying amounts of globulin are the proteins most commonly found in abnormal urine. The original of these ordinary proteins lies in the plasma of blood.

Albumin urine may by divided to three state :

1. Functional albuminuria.

   Increased protein consumption (won pathological cause: pregnancy, physical excretion)(anaerobic exercises) and fevers.
2. False albuminuria.

In this state albumin is present in urine as a result of from urinary traction bleeding.

3. True albuminuria.

In is a state of filtration of some plasma protein through glomerular membrane indicate nephritic syndrome.

Another kind of protein, called "Bence Jones" protein produced by the bone marrow and this is not a plasma protein. Its presence is associated with bone marrow disease (multiple myeloma).

Quantitative tests for urinary protein are generally carried out on 24 hr. urine collection. Normally small amount of protein is excreted in the urine ranging between (0.1-0.2) g / 24 hour greater than this range, indicate proteinuria abnormal levels of protein in urine are an indicator of kidney or urinary tract disease.

**Methods:**

**Materials:**

1. Urine sample.
2. Sulphosalicylic acid (20% SSA).
3. Acetic acid (10%).

**Procedure:**

Before doing the test for protein, it is essential that the urine should be clear and it may be, therefore necessary to filter or centrifuge it.

a. **Sulphosalycilic acid test (SSA):**

It is specific test for protein, that is reliable, simple and does not require heat.

1. Take 5 ml of urine.

2. Add (2-5) drops of 20% SSA.
3. Turbidity indicated the presence of protein.

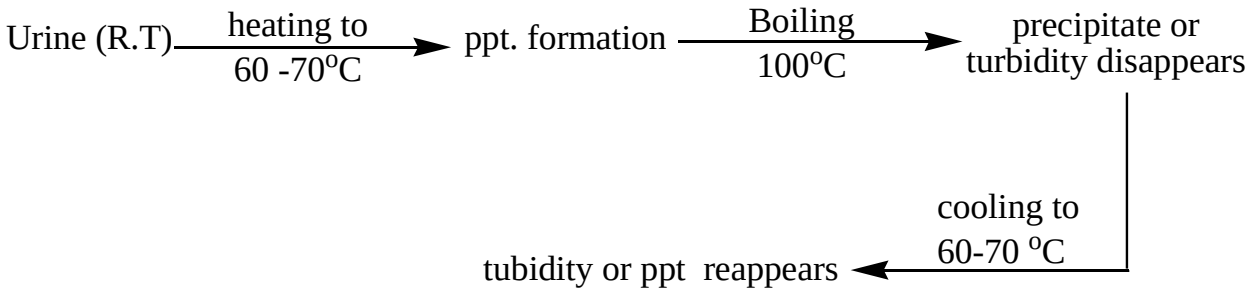
**b. Boiling point test (heattest) heat coagulation:**

Based on precipitation by heat and coagulation by acids. It is less convenient than the sulphosalicylic acid test.

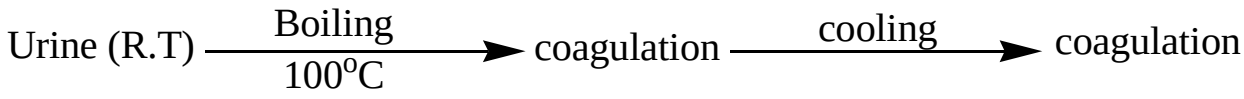
1. Take (5) ml of urine in a test tube, incline the tube at an angle.
2. Boil the top (2) cm over a flame while holding the bottom of the tube, cloudiness due to the presence of protein or phosphate.
3. Add (2-3) drops of acetic acid, if cloudiness disappears it is due to phosphate, and protein is not present.

the presence of Bence-Jones proteinuria. This is confirmed by heating the urine sample to 50°C and the formation of precipitate which disappears on boiling and reappears on cooling may indicate the presence of Bence-Jones protein. This is an abnormal protein that is excreted in urine of patients with multiple myloma, hyperglobulinemia.

**Test for Bence-Jones protein:**




Other proteins don't act as this protein




## 2. Sugars:

A sample of urine which reduce benedict reagent indicates the presence of reducing carbohydrate or substances. These substances are glucose, galactose, fructose, lactose, maltose, ascorbic acid (vit. C), acetyl salicylic acid aspirin or homogentisic acid. Although glucose is easily filtered in the glomerulus, it is not present in the urine because all of the glucose filtered is normally reabsorbed from the renal tubules back into the blood. Presence of glucose in the urine is called glucosuria or glyiosuria. Main cause: diabetes mellitus.

**Method:**

**Benedict test.**

**Materials:**

1. Urine sample.
2. Benedict reagent (100 g sodium carbonate, 173 g sodium citrate, 17.3 g $CuSO_4$ in liter D.W).

**Principle:**

Reduction cupric (blue) to copprous (brick red) by the reducing sugars.

**Procedure:**

Urine must be free of protein which interfere with the reagent used this can be done by heating the sample and then filtered it.

1. To (5) ml of benedict reagent add (0.5) ml (about 8 drops) of urine.
2. Mix by shaking in a water bath.
3. Boil for about (5) min.

4. Allow to cool, turbidity or precipitate indicates the presence of reducing sugar in the following manner:

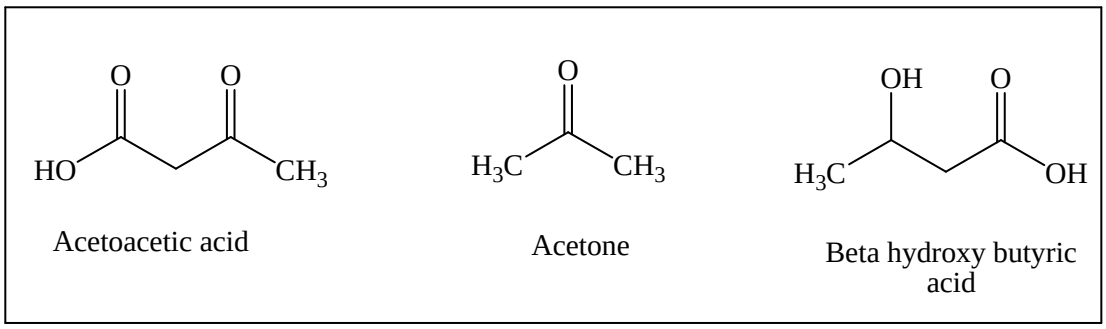
- Light green turbidity (+). 0.1-0.5%
- Green precipitate (++). 0.5-1.0%
- Yellow(+++). 1.0-2.0%
- Red or reddish brown (++++) 2% and over

However a more specific and sensitive quantitative test for glucose is the clinistix method. It is specific for glucose. The reagent strip is dipped in the urine, and the colour of the test area is compared with the marker's colour chart 10 second later.

5. Record your results.

## 3.Ketone bodies:

These are compounds acetoacetic acid, β-hydroxy butyric acid and acetone.




They may appear in the urine of patients with:

1. Severe diabetes mellitus.
2. After starvation and prolonged vomiting.
3. Prolonged fasting.
4. Fevers.
5. After anesthesia, diarrhey.

It forms from the oxidation of free fatty acids (FFA) and lipids in diabetic patient in which glucose can not be oxidize for formation of energy. The ketones may be detected using rothera reagent or one of it's modification such as ketostix, aceto test, lange test.

Increase in ketones body in blood cause decrease of pH so may cause dangerous diabetic coma.

**Methods:**

**a. Rothera's test:**

**Materials:**

1. Urine.
2. Ammonium sulphate (sat.).
3. Sodium nitroprusside (5 L) $Na_2(FeCN)_5NO$ (freshly prepared).
4. Ammonium hydroxide (28%).

**Procedure:**

1. Take (2) ml of urine.
2. Add ammonium sulphate (sat.).
3. Shaking, add (4-5) drops of sodium nitroprusside.
4. Add (1) ml of $NH_4OH$.
5. Mix, allow to stand for 5 min. record your results. (deep permanganate colour is appear).

**b. Lange test:**

**Materials:**

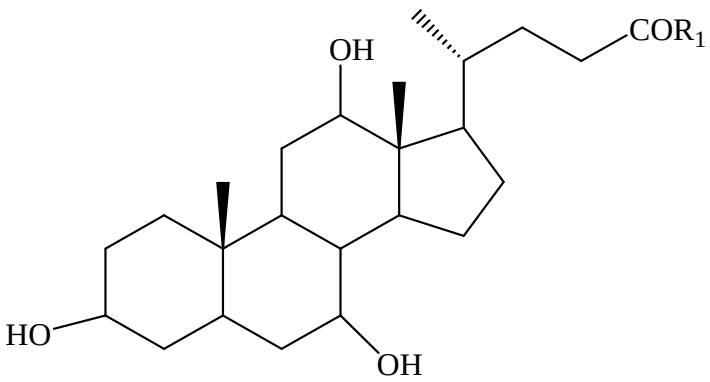
1. Urine.
2. Glacial acetic acid.
3. Na-nitroprusside, freshly prepared concentrated sol.

**Procedure:**

1. Take (2) ml of urine.
2. (3) drops of glacial acetic acid.
3. Add (4-5) drops of Na-nitroprusside.
4. Red ring indicate the presence of ketone body.

## 4.Bile salts and bile acids:

Bile acids form from the oxidation of cholesterol in liver after that it store in gallbladder used in the factor for the digestion and absorption of diet triglyceride.




$R_1$ = OH (cholic acid)

$R_1$ = H (Deoxycholic acid)

$R_1$ = glycine (Glycocholic acid)

$R_1$ = Taurine (taurocholic acid)

$\xrightarrow{Na^+}$ Bile salts

**Clinical significance of bile salts:**

1. Bile salts dissolve triglyceride and free fatty acids by forming micells with them and cause emulsion in the intestine.
2. Bile acids with phospholipids dissolve cholesterol in the gallbladder so prevent formation of gallstones.

3. Formation of bile acids from cholesterol decrease the amount of cholesterol in blood.
4. Excreted large amount of waste products like medicals, poisonus materials, some salts (Cu, Zn, Hg).
5. Increase the the absorption of lipid dissoluble vitamine A, E, D,K.

**Test for bile salts:**

**Methods:**

a. **Millios test**

**Materials**

1. Urine.
2. (0.1%) furfural sol.
3. Conc. $H_2SO_4$.

**Procedure:**

1. Take (5) ml of urine.
2. Add 5 drops of furfural sol.
3. Mix well.
4. Add 3 ml of conc. $H_2SO_4$.
5. Cooling, mix well.
6. Red sol. Indicate the presence of bile salts.

b. **Hay's test**

This test is based upon the principle that bile acids have the property of reducing the surface tension of fluids in which contained.

**Materials:**

1. Urine.
2. Dry sulfur.

**Procedure:**

1. Take small amount of urine (about 10 ml) in the test tube.
2. Sprinkle finely a little of dry sulfur over the surface of the urine from a height.
3. If bile salts are present, sulphur will sink to the bottom, if bile salts are absent, sulphur will float on the surface.

## 5.Bilirubin:

In health, bilirubin is not found in the urine the finding of bilirubinuria in a jaundiced patient suggest that the jaundice is due to the appearance of conjugated bilirubin in the plasma which could be due to either hepatocellular damage or hepatic obstruction.

**Test's for bilirubin:**

1. **Foam test.**

**Principle:**

Bitirubin if present colours of the foam yellow to green.

**Procedure:**

1. place (5) ml of urine in a test tube. Place cover.
2. shake the urine vigorously for 3 min.

If bilirubin is present, the foam produced will have a yellow to green colour.

**2. Fouchet's test"**

**Materials:**

1. Urine.
2. Barium chloride (10%).
3. Fouchet's reagent (TCA 25 g, D.W 100 ml) 10 ml ferric chloride.

**Procedure:**

1. Add a (2-5) ml of barium chloride solution to about (10) ml of urine in a test tube.
2. Filter and allow to drain well.
3. Sepread the filter paper on another dry paper and add drop or two of fouchet's reagent.
4. A greenish – blue colour due to an oxidation product of bilirubin is obtained for a positive test.

**Note:** commercial products based on the diazo reaction are available in reagent strips or tablets.

**Clinical significance:**

Bilirubin is found in urine of patients with obstructive jaundice. Also bilirubinuria may be found in early stage of viral hepatitis.

## 6.Urobilinogen:

Urobilinogen, being colourless, is not apparent on inspection unless it has been converted on standing to urobilin which gives an orange brown colour to the urine. Small amount of Urobilinogen is excreted normally in the urine of healthy person. Excess urobilinoginuria may occur during hemolysis of red cells which may be insufficient to cause clinical. Jaundice, or it may occur in the preicteric stage of infective hepatitis and in diffuse liver disease as liver cirrhosis.

**Test of Urobilinogen:**

Fresh sample is needed because is oxidized on exposure to air. Red colour is given by p-dimethyl aminobenzaldehyde (Ehrlich's aldehyde reagent) in strong acid.

**Ehrlich test:**

**Reagents:**

1. Ehrlich's reagent: dissolve 2 g of p-dimethylamino –benzaldehyde in (100) ml of 20% HCl.

**Procedure:**

1. Add to (5) ml freshly voided urine, to (0.5) ml of Ehrlich's aldehyde reagent.
2. Allow to stand (3-5) min and note any colour preduced.
3. Compare with a blank using (5) ml of the urine + 1 ml of (6 mol/L) HCl, because acidification of urine changes its colour. Pink colour is observed cherry red color when abnormally high amount.

**Note:** commercial strips are available and should be used on freshly voided urine.

**Clinical significance:**

Urobilinogen is raised in haemolytic jaundice and in all forms of retention jaundice. In liver cirrhosis, although less Urobilinogen is formed in the intestine impaired liver function of ten results in an

appreciable increase in urine Urobilinogen. In this case biliuribin may not be present in the urine.

## 7.Blood:

Blood may be present in urine as intact red blood cells which may be seen under the microscope or as hemoglobin resulting from the haemolysis of red blood cells.

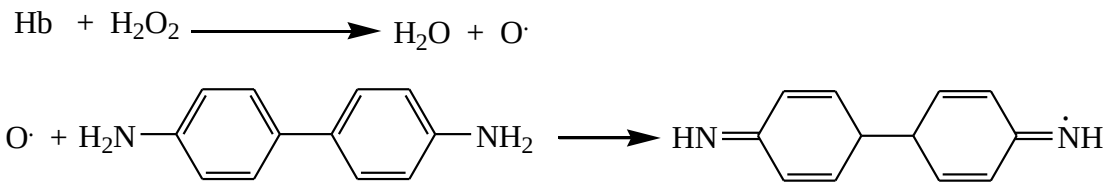
There is two method for detection of blood in urine:

1. Chemical method (Benzidine test).
2. Microscopical method.

**Benzidine test:**

Is highly sensitive.

**Principle:**




Colorless benzidine

blue colored

**Material:**

1. Urine sample.
2. Benzidine reagent 4 g benzidine, 100 ml glacial acetic acid.
3. (3%) $H_2O_2$.

**Procedure:**

1. Take (2) ml of urine in a test tube.
2. Add (1) ml of benzidine reagent.
3. Add (1) ml of $H_2O_2$ to the mixture.

4. Mix well, green or blue colour indicate the presence of blood in the urine (Haematuria).

## Microscopical examination

Examination of urine sediment may reveal the presence of different type of cells such as epithelial cells, leukocgtes, erythrocytes, or renal cells. Different types of crystals, yeast, bacteria, or casts may also be present.

Fresh urine should be examined as some urinary findings may disappear on standing. Urine which is turbid (when passed or on storage in a refrigerator) interferes with microscopic examination.

The reaction of urine may alter the objects to be seen under the microscope.

**Procedure (1):**

Deposite allowed to settle down by gravity in a conical receiver,

**Procedure (2):**

1. Transfere urine sample (5) ml to a centrifuge tube.
2. Centrifuge your sample at a moderate speed for 5 minutes, be sure balance centrifuge.
3. Discard the supernatant (fluid off the top) by quickly pouring of fluid.
4. Mix sediment with remaining fluid.
5. Transferring 1 drop of material to a slide and covering with a cover slip.
6. Examine the sample under the microscope under low and high power, fiald (LPF & HPF).

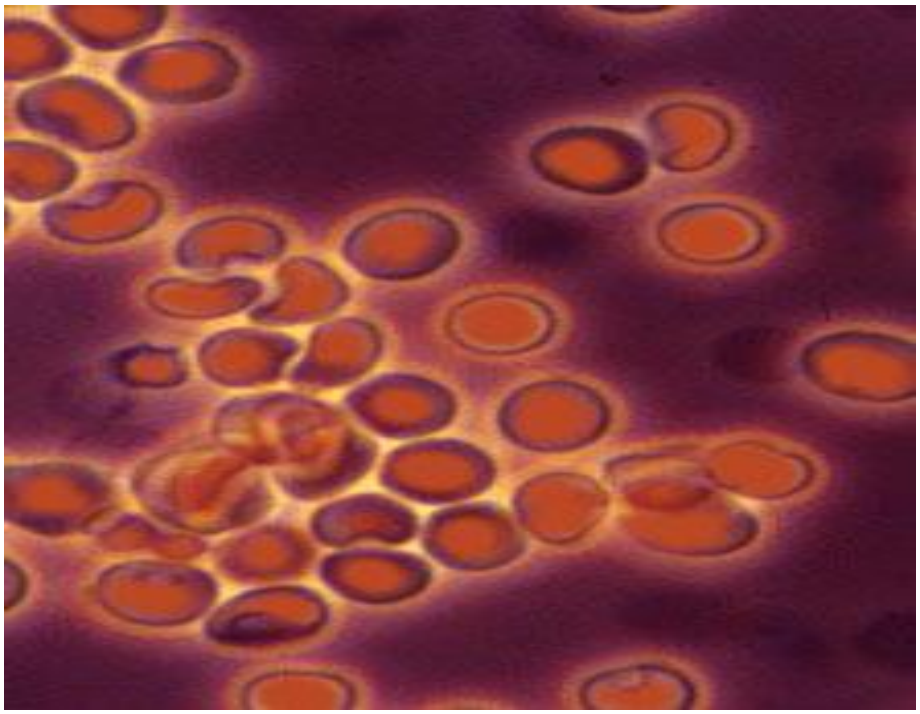
7. Identify what you see by comparing to charts. Draw a few of your observations.

**Urinary deposits are divided into :**

- **Organized**

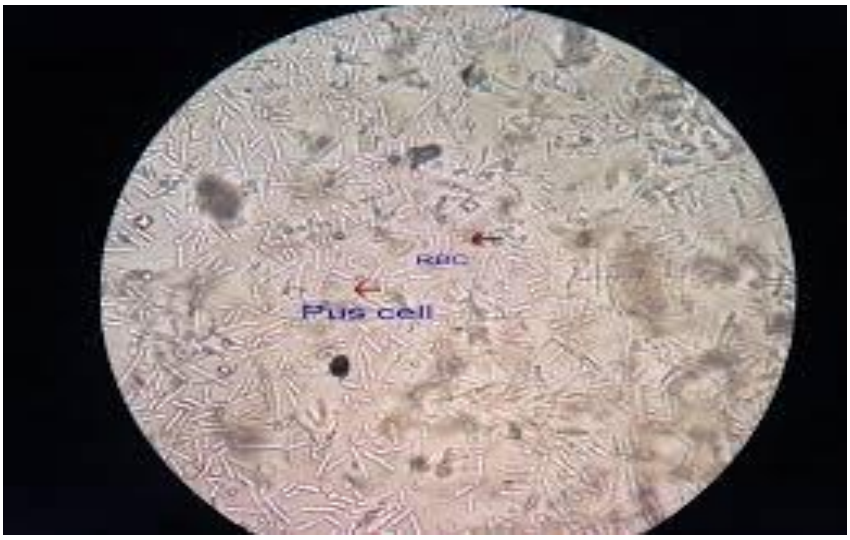
**a. Red blood cells:**

Normal centrifuged urine contains < (1) RBC / HPF (high power field).The RBCS appear as roughly circular element with clear yellowish centers. The detection of microscopical haematuria may be very important in the diagnosis, particularly in patients with systematic diseases. The presence of RBCs with pus cells is usually seen in urinary tract infection (UTI).



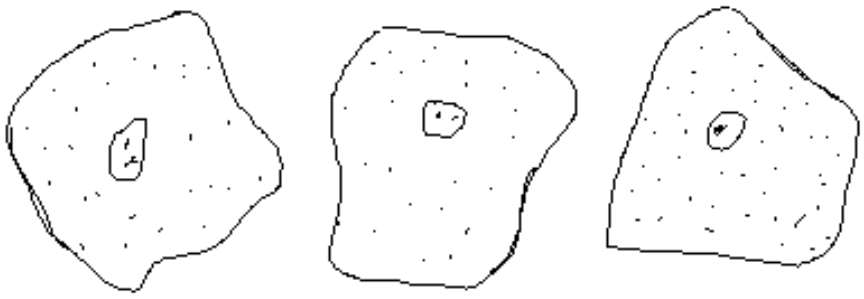
**b. Pus cells:**

Normal urine contains no or few pus cells. The presence of pus cells more than 10/HPF in urine is abnormal in adult women and it indicates urinary tract infection. The presence of pus cells more than 3/HPF is significant in adult men.

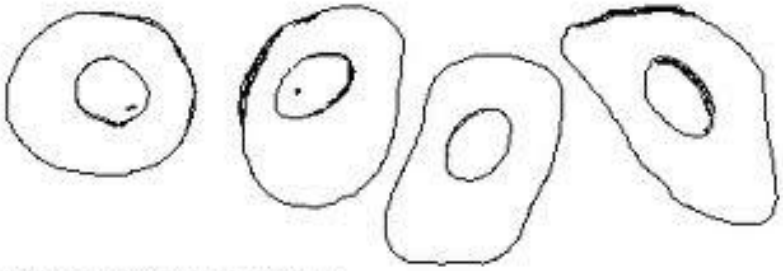



**c. Epithelial cells:**

Transitional epithelial cells from the bladder or ureters appear as large oral cells or sheets of polygonal cells. It is a normal lining of the urinary tract.



**Squamous Epithelial Cells**



Transitional Epithelial Cells

**d. Casts:**

They are formed due to the precipitation of mucoproteins in the renal tubule.

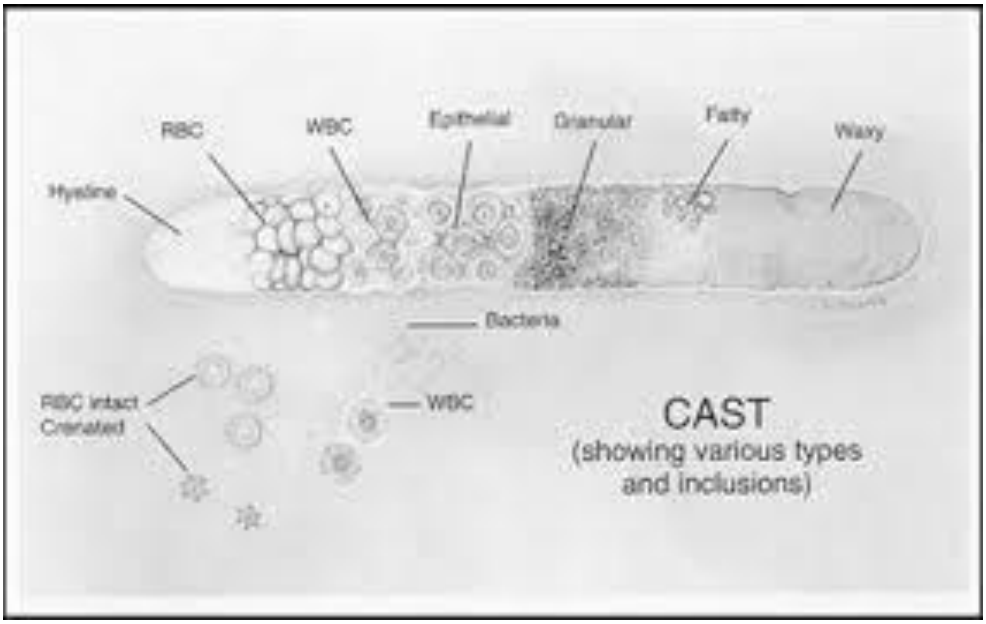
**1.** Hyaline casts are pale colourless. Soluble in water, transparent and homogenous. They are found in chronic glomerulonephritis and occasionally in small number of normal urine. Hyaline casts are usually caused by dehydration, exercise, or diaretic medicines.

- **Granular casts:**

They are formed as aresult of disintegration of cellular elements (fats or protein). It is tubular in shape with nuclei. It contains fine or course granules.

Granular Cast

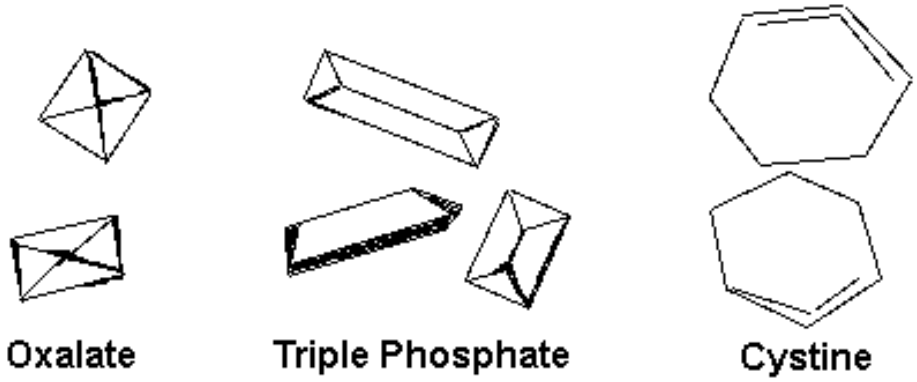
The presence of granular cast always indicates renal demage and it's appearance is indicative of inflammation and degeneration of the renal tubules.

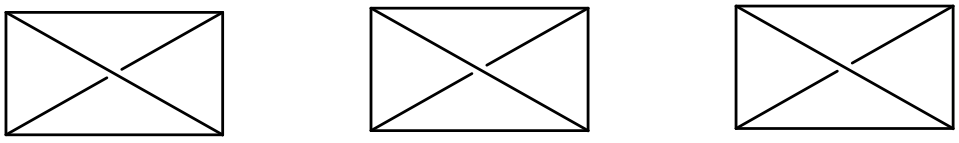



- **Unorganized (crystals):**

**a. Calcium oxalate:**

This is the most common type of crystals seen. It is characterized by it's envelop like shape as single or often grouped in the form of rosette.

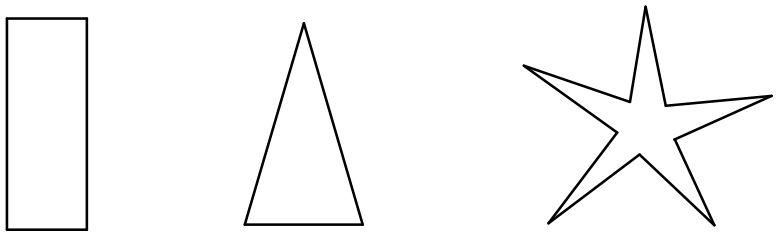


Calcium oxalate crystals are normal in acidic urine. Excess of oxalate might lead to stone formation.

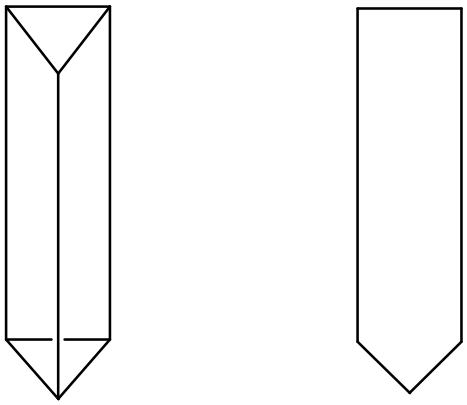
**b. Uric acid:**

Yellow to reddish brown prisms or it could be in the from of rosette present in acidic urine.



**c. Triple phosphate:**

Colourless prisms or feathery stars usually seen in alkaline urine.



**d. Amorphous urate (phosphate):**

Yellow to reddish brown particles with sharp needle like projection.

Chemical deposits:

| | |
|---|---|
| Acidic urine | Alkaline urine |
| Calcium oxalate | Amorphous phosphates |
| Uric acid | Stellar phosphates |
| Urates (amorphous) | Triple phosphates |
| | Ammonium urate |

Tyrosine, xanthine, hippuric acid, leucine, sulphonamides, miscellaneous-mucus, spermatozoa, bacteria, parasites.

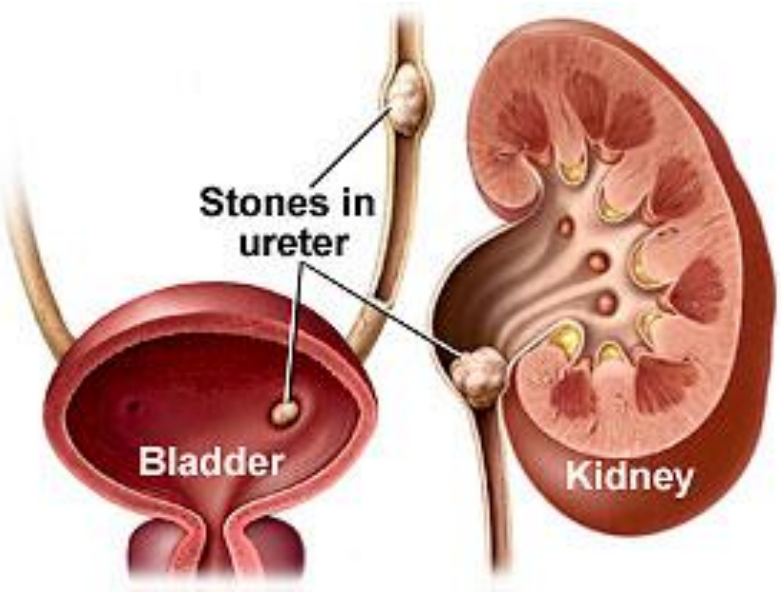
**Note:**

High protein diets increase acidity. Vegetarian diets and bacterial infections increase alkalinity.

## calculus

Calculus (also named stones)an abnormal concertion formed in body tissiues by an accumulation of mineral salts, also composed of metabolic products present in glomerular filtrate. Calculi are usually found in biliary and urinary tracts. Kinds of calculi include biliray calculus and renal calculus.

Kidney stone a hard crystalline material formed in renal tubules ureter or bladder. It can be as small as agrain of sand or as large as a golf ball.




## Conditions causing kidney stone:

1. High concentration of metabolic products in glomerular filtrate.
2. Changes in urine pH due to :

- Bacterial infiction.
- Precipitation of salts at defferent pH.

3. Obstruction of urinary flow.
4. Deficiency of ***stone-forming inhibitors*** in urine. (citrate, pyrophosphate, glycoproteins inhibit growth of calcium phosphate and calcium oxalate crystals)
5. Genetics.
6. Estrogen.
7. Cholesterol lowering drugs.
8. Diabetes.
9. Rapid weight loss.
10. Dietary risk factors..

## Dietary risk factors associated with increased:

- Stone risk:

1. Low fluid intake.
2. High intake of animal protein.
3. High dietary sodium.
4. Excessive intake of refined sugars.
5. Food rich in oxalate.
6. High intake of grape fruit juice, apple juice and soft cola drinks.
7. Family history of kidney stones (increase risk by three times).
8. History of hypertention.
9. Obesity.

## Types of kidney stones:

1. Calcium salts.
2. Uric acid.
3. Mg ammonium $PO_4$ ($NH_4MgPO_4.6H_2O$).
4. Cystine.
5. Others (Xanthine, etc.).

1. Calcium stones (alone or in combination) the most common types of urinary stones. 80% of kidney stones contain calcium (calcium oxalate, calcium phosphate, calcium carbonate).

The type of salt depends on:

- Urine pH.
- Availability of oxalate (Hyper oxaluria).

2. Uric acid stones: urinary calculus formed from uric acid is more common in men than in women. About (8)% of renal stones contain of uric acid form in acidic urine, occur in people with gout or those going through chemotherapy.
3. Mg ammonium $PO_4$ (struvite) kidney stones.

- About (10)% of all renal stones contain Mg amm. $PO_4$.

- Associated with chronic urinary tract infection (microorganisms that metabolize urea into ammonia, causing urine pH to become alkaline and stone formation).

**4.** Cystine stones.

- A rare less than 1% of kidney stones.
- Due to homozygous cystinuria.
- Form in acidic urine.
- Soluble in alkaline urine.

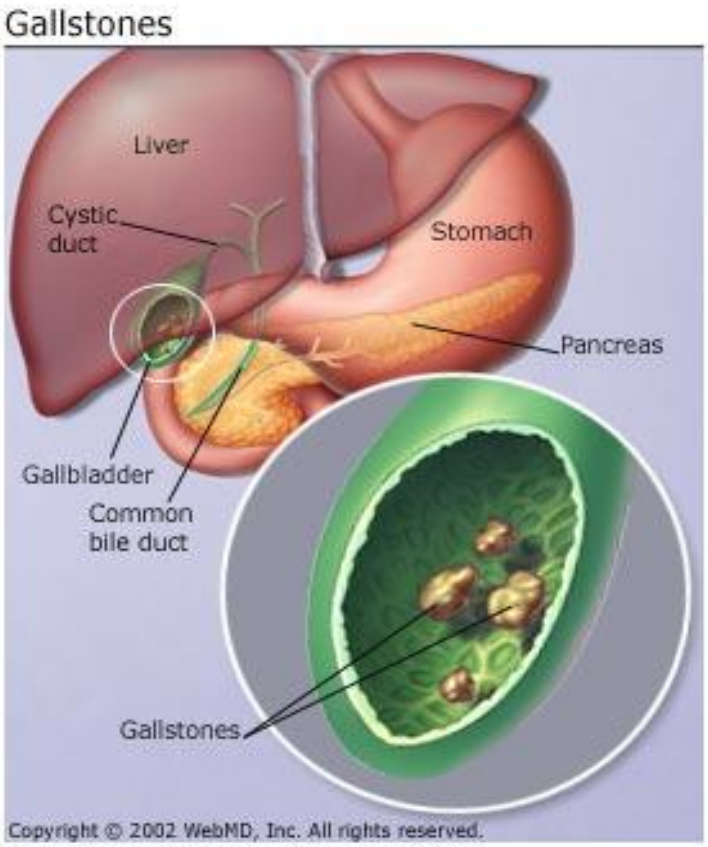
## Biliary calculi

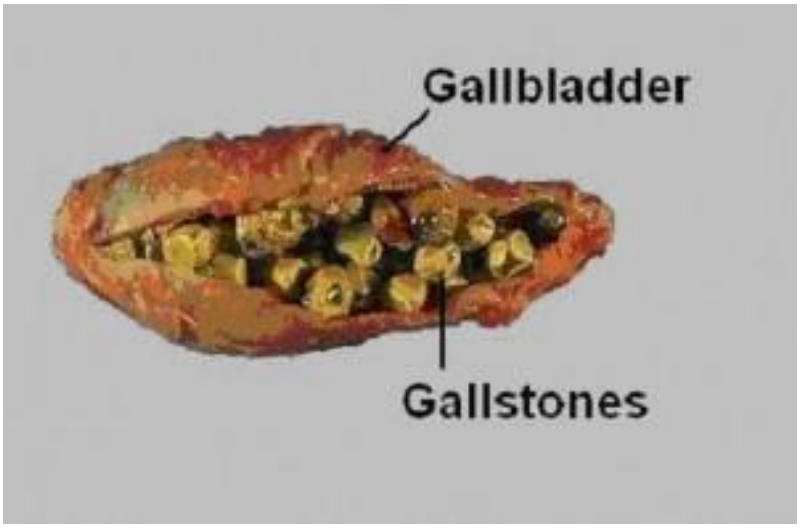
## (Gallstones)

### Gallstones:

Form in the gallbladder, a small organ located under the liver. The gallbladder acids in the digestive process by storing bile and secreting it into the small intestine when food enters.

Bile is a fluid produced by the liver and is made up of several substances including cholesterol, bilirubin, and bile salts.




Gallstones are pieces of solid material that form in the gallbladder. These stones develop because cholesterol and pigments in bile sometimes form hard particles, the two main types of gallstones are:

- Cholesterol stones:

- Pigment stones:

Several factors may come together to create gallstones including

- Genetics.
- Body weight.
- Decreased motility (movement) of the gallbladder.
- Diet type.
- Increase incon. Of cholesterol in bile change in pH

Gallstones can form when there is an imbalance in the substances that make up bile.

**Kidney stone analysis:** is a test done on a kidney stone if stone has formed and removed chemical analysis of stone helps to:

1. Identify the cause.
2. Find the chemicals make up to a kidney stone.
3. Guide treatment for a kidney stone.
4. Give information on how to prevent more kidney stone formation.

## Physical characteristics: of a stone

1. Size.
2. Shape.
3. Weight.
4. Color.
5. Texture.

## Materials:

- Calculi powder.
- 1 N $HNO_3$.
- 4% ammonium oxalate.

- 5N ammonium solution (28.6 ml ammonium hydroxide ⟶ 100ml distilled water).
- 5% ammonium molybdate sol.
- 0.5% Vit. C.
- 2.5% calcium chloride.
- 1N KOH (5.6 g/100ml).
- 10% Folin solution.
- 1% sodium nitroprusside ($Na_2(FeCN)_5NO$) freshly prepared.
- Filter paper.
- pH paper.

## Procedure:

1. wash the calculi with distilled water.
2. Dry it in oven.
3. Mill, with mortar and pastle.
4. Take small amount of the powder in test tube.
5. Add 5 ml of (1 N) $HNO_3$, effervescence indicate the presence of carbonate ($CO_3^{=}$).
6. Put the test tube in boiling water bath for 5 min.
7. Cool and filtered (use filter paper).
8. Divide the filtrate to three parts.

| 1st Part | 2nd Part | 3rd Part |
|---|---|---|
| Test for $Ca^{++}$, $Mg^{++}$ | Test for $PO_4^{\equiv}$ | Test for oxalate ($C_2O_4^{=}$) |
| **1.** Add 1 ml of ammonium oxalate. | **1.** Add 1 ml of ammonium molybdate sol. | **1.** Add 1 ml of $CaCl_2$ or 1 ml $BaCl_2$ |
| **2.** Adjust the pH (5) by KOH or $KH_2PO_4$ | **3.** Add 1 ml of Vit. C. Blue ppt. indicate | **2.** Adjust the pH 5 by KOH or $KH_2PO_4$. |

| | | |
|---|---|---|
| **3.** Formation of white precipitation indicate the presence of Ca ($CaC_2O_4$) calcium oxalate. | presence of phosphate | **3.** White ppt. indicate the presence of oxalate. |
| **4.** Filtered the ppt. | | |
| **5.** To the supernatant add 1 ml of ammonium solution. | | |
| **6.** Add 1 ml of $KH_2PO_4$ solution. | | |
| **7.** Whit ppt. indicate the presence of Mg. | | |

**Blood**