URINE ANALYSIS

COMPOSITION OF NORMAL URINE

	Parameters	Values
1.	Volume	600-2000 ml
2.	Specific gravity	1.003-1.030
3.	Osmolality	300-900 mOsm/kg
4.	pH	4.6-8.0
5.	Glucose	<0.5 gm
6.	Proteins	<150 mg
7.	Urobilinogen	0.5-4.0 mg
8.	Porphobilinogen	0-2 mg
9.	Creatinine	14-26 mg/kg (men), 11-20 mg/kg (women)
10.	Urea nitrogen	12-20 gm
11.	Uric acid	250-750 mg
12.	Sodium	40-220 mEq
13.	Potassium	25-125 mEq
14.	Chloride	110-250 mEq
15.	Calcium (low calcium diet)	50-150 mg
16.	Formiminoglutamic acid (FIGlu)	< 3 mg
17.	Red cells, epithelial cells, and white blood cells	<1-2/high power field

INDICATIONS FOR URINALYSIS

1. Suspected renal diseases like glomerulonephritis, nephrotic syndrome, pyelonephritis, and renal failure.

2. Detection of urinary tract infection.

3. Detection and management of metabolic disorders like diabetes mellitus.

4. Differential diagnosis of jaundice.

5. Detection and management of plasma cell dyscrasias.

6. Diagnosis of pregnancy.

COLLECTION OF URINE

- **First morning, midstream**: Preferred for routine urine examination.
- **Random, midstream**: Routine urine examination.
- **First morning, midstream, clean catch**: Bacteriological examination.
- **Postprandial**: Estimation of glucose, urobilinogen
- **24-hour**: Quantitative estimation of proteins or hormones.
- **Catheterised**: Bacteriological examination in infants, bedridden patients, and in obstruction of urinary tract.
- **Plastic bag** (e.g. colostomy bag) tied around genitals: Infants, incontinent adults.

CHANGES WHICH OCCUR IN STANDING URINE@ROOM TEMPERATURE

- **Increase in pH due to production of ammonia from** urea by urease-producing bacteria.
- **Formation of crystals due to precipitation of phosphates** and calcium (making the urine turbid)
- **Loss of ketone bodies, since they are volatile.**
- **Decrease in glucose due to glycolysis and utilization** of glucose by cells and bacteria.
- **Oxidation of bilirubin to biliverdin causing false-negative** test for bilirubin
- **Oxidation of urobilinogen to urobilin causing false-negative** test for urobilinogen
- **Bacterial proliferation**
- **Disintegration of cellular elements, especially in** alkaline and hypotonic urine.

Urine sample must be tested in the laboratory within 2 hours of collection to get the correct results.

PRESERVATION OF URINE SAMPLE

- **Refrigeration (4-6°C)** is the best general method of preservation of urine sample up to 8 hours.
- Following chemical preservatives can be added to the 24-hour urine sample:
  - **Hydrochloric acid**: It is used for preservation of a 24- hour urine sample for adrenaline, noradrenaline, vanillylmandelic acid, and steroids.
  - **Toluene**: It forms a thin layer over the surface and acts as a physical barrier for bacteria and air. It is used for measurement of chemicals.
  - **Boric acid**: A general preservative.
  - **Thymol**: It inhibits bacteria and fungi.
  - **Formalin**: It is an excellent chemical for preservation of formed elements.

URINE EXAMINATION

- **PHYSICAL EXAMINATION**
- **CHEMICAL EXAMINATION**
- **MICROSCOPIC EXAMINATION**

PHYSICAL EXAMINATION
Volume

- Average 24-hr urinary output in adults is 600-2000 ml.
- The volume varies according to fluid intake, diet, and climate.
- Abnormalities of urinary volume are as follows:
  - **Polyuria means urinary volume > 2000 ml/24 hours.**

    This is seen in diabetes mellitus, diabetes insipidus, chronic renal failure, diuretic therapy.
  - **Oliguria means urinary volume < 400 ml/24 hours.**

    This is seen in febrile states, acute glomerulonephritis, congestive cardiac failure or dehydration.
  - **Anuria means urinary output < 100 ml/24 hours or complete cessation of urine output.**

    This occurs in acute tubular necrosis (e.g. in shock, hemolytic transfusion reaction), acute glomerulonephritis and complete urinary tract obstruction.

PHYSICAL EXAMINATION
Color

- Normal urine color in a fresh state is pale yellow or amber and is due to the presence of various pigments collectively called urochrome.

Colors	*Conditions*
Colorless	Dilute urine (diabetes mellitus, diabetes insipidus, overhydration)
Red	Hematuria, Hemoglobinuria, Porphyria, Myoglobinuria
Dark brown or black	Alkaptonuria, Melanoma
Brown	Hemoglobinuria
Yellow	Concentrated urine
Yellow-green or green	Biliverdin
Deep yellow with yellow foam	Bilirubin
Orange or orange-brown	Urobilinogen Porphobilinogen
Milky-white	Chyluria
Red or orange fluorescence with UV light	Porphyria

PHYSICAL EXAMINATION

Appearance

- Normal, freshly voided urine is clear in appe
- Foamy urine occurs in the presence of excess proteins or bilirubin.

Cause	*Appearance*	*Diagnosis*
1. Amorphous phosphates	White and cloudy on standing in alkaline urine	Disappear on addition of a drop of dilute acetic acid
2. Amorphous urates	Pink and cloudy in acid urine	Dissolve on warming
3. Pus cells	Varying grades of turbidity	Microscopy
4. Bacteria	Uniformly cloudy; do not settle at the bottom following centrifugation	Microscopy, Nitrite test

PHYSICAL EXAMINATION

Odor

- Freshly voided urine has a typical aromatic odor due to volatile organic acids.
- After standing, urine develops ammoniacal odor → formation of ammonia occurs when urea is decomposed by bacteria).
- Some abnormal odors with associated conditions are:
  - **Fruity: Ketoacidosis, starvation**
  - **Mousy or musty: Phenylketonuria**
  - **Fishy: Urinary tract infection with *Proteus, tyrosinaemia.***
  - **Ammoniacal: Urinary tract infection with *Escherichia coli, old standing urine.***
  - **Foul: Urinary tract infection**
  - **Sulfurous: Cystinuria**

PHYSICAL EXAMINATION
Specific Gravity (SG)

- Measure of concentrating ability of kidneys and is determined to get information about this tubular function.
- It is basically a comparison of **density of urine** against the density of distilled water at a particular temperature.
- **Normal SG of urine is 1.016 to 1.022** in 24 hrs sample and also depends on the state of hydration.
- SG of normal urine is mainly related to urea and sodium.
- SG increases **as solute concentration increases** and decreases when temperature rises (since volume expands with rise in temperature).

PHYSICAL EXAMINATION
Specific Gravity (SG)

- **Hypersthenuria -** Causes of increase in SG of urine are diabetes mellitus, glycosuria, albuminuria, fever and dehydration.
- **Hyposthenuria -** *Causes of decrease in SG of urine are diabetes insipidus,* pyelonephritis, diuretics and alcohol
- **Isosthenuria -** Low and fixed SG at 1.010 due to loss of concentrating ability of tubules seen in end stage renal failure.

Methods for measuring SG are urinometer method

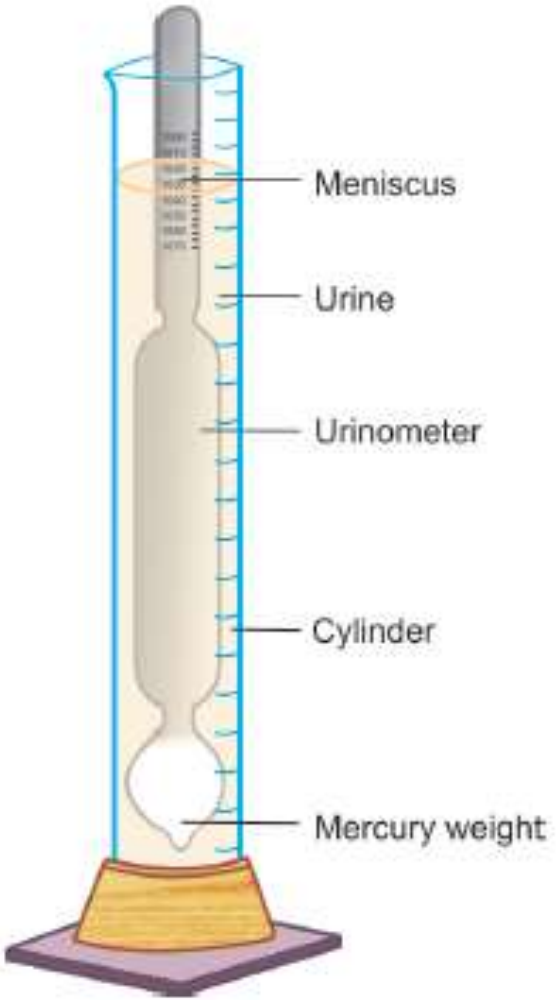
PHYSICAL EXAMINATION
Specific Gravity (SG)

Urinometer

PHYSICAL EXAMINATION
Specific Gravity (SG)

Urinometer

1. Fill a measuring cylinder with 50 ml of urine.
2. Lower urinometer gently into the urine and let it float freely.
3. Let urinometer settle; it should not touch the sides or bottom of the cylinder.
4. Take the reading of SG on the scale (lowest point of meniscus) at the surface of the urine.
5. Take out the urinometer and immediately note the temperature of urine with a thermometer.
6. *For every 3°C increase / decrease add / subtract 0.001*



PHYSICAL EXAMINATION
Specific Gravity (SG)

Refractometer method:

- SG can be precisely determined by a refractometer, which measures the refractive index of the total soluble solids.
- Higher the concentration of total dissolved solids, higher is the refractive index.
- Extent of refraction of a beam of light passed through urine is a measure of solute concentration, and thus of SG.

• The method is simple and requires only 1-2 drops of urine.
• Result is read from a scale or from digital display.

PHYSICAL EXAMINATION
Specific Gravity (SG)

Reagent strip method:

- Measures the concentration of ions in urine, which correlates with SG.
- Depending on the ionic strength of urine, a polyelectrolyte will ionize in proportion.
- This causes a change in color of ph indicator (bromothymol blue).

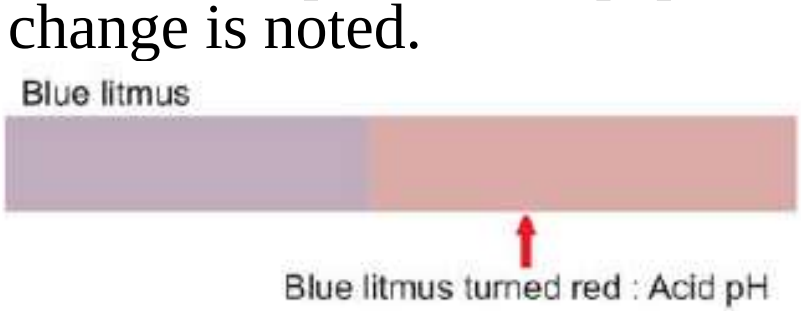
PHYSICAL EXAMINATION
Reaction and pH

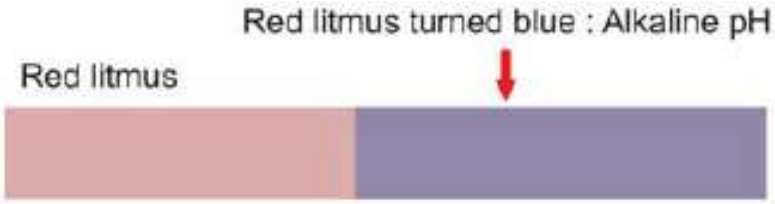
- pH is the scale for measuring acidity or alkalinity.
- Fresh urine is required for correct estimation of pH → upon standing, urine becomes alkaline owing to loss of CO_2 & production of ammonia from urea.
- Normal value ranges from 4.6-8
- Urine pH depends on diet, acid base balance, water balance, and renal tubular function
- Various methods for determination of reaction of urine:
  - Litmus paper
  - pH indicator paper
  - pH meter
  - Reagent strip tests

PHYSICAL EXAMINATION
Reaction and pH

1. Litmus paper test:

- A small strip of litmus paper is dipped in urine and any color change is noted.





2. pH indicator paper:

- Reagent area (impregnated with bromothymol blue and methyl red) of indicator paper strip is dipped in urine sample and the color change is compared with the color guide provided.
- Approximate pH is obtained.

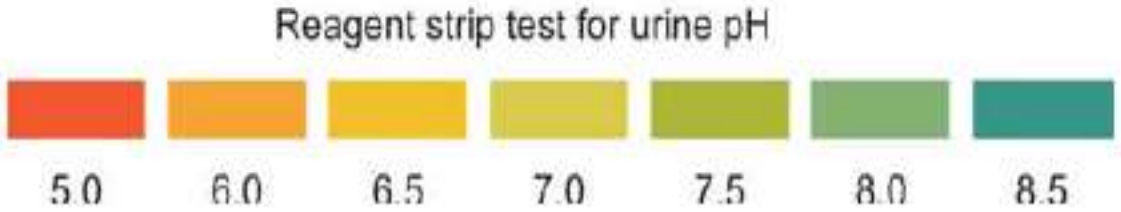
PHYSICAL EXAMINATION
Reaction and pH

3. pH meter:

- An electrode of pH meter is dipped in urine sample and pH is read off directly from the digital display.
- It is used if exact pH is required.

4. Reagent strip test:

- The test area contains polyionic polymer bound to H^+
- On reaction with cations in urine, H+ is released causing change in color of the pH-sensitive dye.



PHYSICAL EXAMINATION
Reaction and pH

Acidic urine

- Ketosis
  - diabetes mellitus
  - Starvation
  - fever
- Urinary tract infection by *Escherichia coli*
- *High protein diet.*

Alkaline urine

- *Severe vomiting*
- Old ammoniacal urine sample
- Chronic renal failure
- Urinary tract infection by bacteria that split urea to ammonia - *proteus or pseudomonas*
- Vegetarian diet

URINE EXAMINATION

- **PHYSICAL EXAMINATION**
- **CHEMICAL EXAMINATION**
- **MICROSCOPIC EXAMINATION**

CHEMICAL EXAMINATION

The chemical examination is carried out for the following substances :-

- Proteins
- Glucose
- Ketones
- Bilirubin
- Bile salts
- Urobilinogen
- Blood
- Hemoglobin
- Myoglobin
- Nitrite or leukocyte esterase

CHEMICAL EXAMINATION
Proteins

- Normally, kidneys excrete scant amount of protein in urine

(up to 150 mg/24 hours)

- These proteins include
  - proteins from plasma (albumin)
  - proteins derived from urinary tract
    - Tamm-Horsfall protein*
    - secretory IgA
    - proteins from tubular epithelial cells, leucocytes and other desquamated cells.
- This amount of proteinuria cannot be detected by routine tests.

*normal mucoprotein secreted by ascending limb of the loop of Henle.

CHEMICAL EXAMINATION
Proteins

- **Proteinuria refers to protein excretion in urine >150 mg/24 hours in adults.**
- Causes
  - **Glomerular proteinuria** due to increased permeability of glomerular capillary wall.

    e.g. nephrotic syndrome.
  - **Tubular proteinuria** due to excretion of low molecular weight proteins which are actively reabsorbed by proximal renal tubules in diseased conditions of tubules.

    e.g. acute and chronic pyelonephritis, heavy metal poisoning, tuberculosis of kidney, interstitial nephritis, cystinosis, Fanconi syndrome and rejection of kidney transplant.

CHEMICAL EXAMINATION
Proteins

- Causes
  - **Overflow proteinuria**: When concentration of a low molecular weight protein rises in plasma, it "overflows" from plasma into the urine.

    e.g immunoglobulin light chains or Bence Jones proteins (plasma cell dyscrasias), hemoglobin (intravascular hemolysis), myoglobin (skeletal muscle trauma) & lysozyme (acute myeloid leukemia type M4 or M5).
  - **Hemodynamic proteinuria**: Alteration of blood flow through the glomeruli causes increased filtration of proteins.

e.g high fever, hypertension, heavy exercise**,** congestive cardiac failure, seizures,& exposure to cold**.**

- Post-renal proteinuria: This is caused by inflammatory or neoplastic conditions in renal pelvis, ureter, bladder, prostate, or urethra.

CHEMICAL EXAMINATION
Test for Proteins

- **Heat and acetic acid test**

Principle –

Proteins are denatured & coagulated upon heating to give white cloud precipitate.

Method –

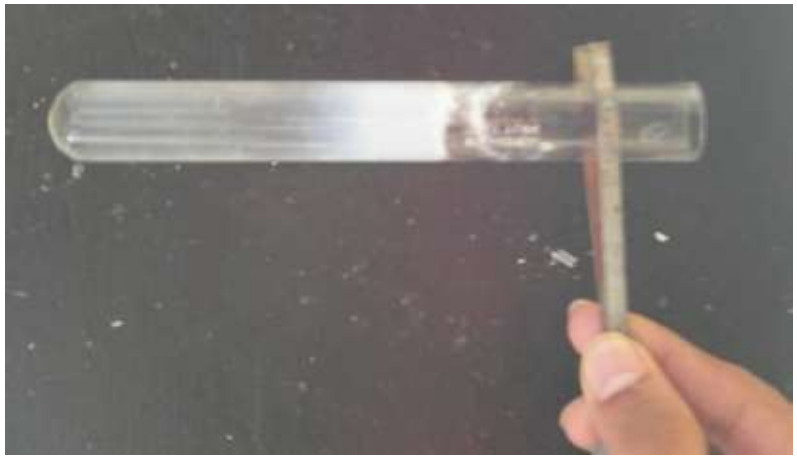
- Take a 5 ml test tube.
- Fill 2/3rd with urine.
- Acidify by adding a few drops of 3% acetic acid if urine is alkaline.
- Boil upper portion for 2 minutes (lower part acts as control).
- If precipitation or turbidity appears, add a few drops of 3% acetic acid.

CHEMICAL EXAMINATION
Test for Proteins

Heat and acetic acid test

Interpretation.

- If turbidity or precipitation disappears on addition of acetic acid, it is due to phosphates.
- if turbidity or precipitation persists after addition of acetic acid, then it is due to proteins.
- The test is semiquantitative and can be graded from traces to 4+ depending upon amount of protein.



No cloudiness	=	Negative
Faint cloudiness	=	Traces (less than 0.1 g/dl).
Cloudiness without granularity	=	+(0.1 g/dl).
Granular cloudiness	=	++(0.1-0.2 g/dl)
Precipitation and flocculation	=	+++(0.2-0.4 g/dl).
Thick solid precipitation	=	++++ (≥ 0.5 g/dl).

CHEMICAL EXAMINATION
Test for Proteins

- **Sulphosalicylic Acid Test**

Principle –

- Addition of sulphosalicylic acid to the urine causes formation of a white precipitate if proteins are present.

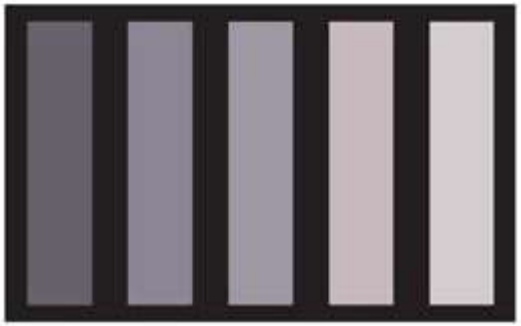
Method –

- Take 2 ml of clear urine in a test tube.
- If reaction of urine is neutral or alkaline, a drop of glacial acetic acid is added.
- Add 2-3 drops of sulphosalicylic acid (3 to 5%) and examine for turbidity against a dark background

Interpretation.

Appearance of turbidity which persists after heating indicates presence of proteins

Sulphosalicylic acid test for proteins in urine



CHEMICAL EXAMINATION
Test for Proteins

- **Heller's Test**

Method –

- Take 2 ml of concentrated nitric acid in a test tube.
- Add urine drop by drop by the side of test tube.

Interpretation-

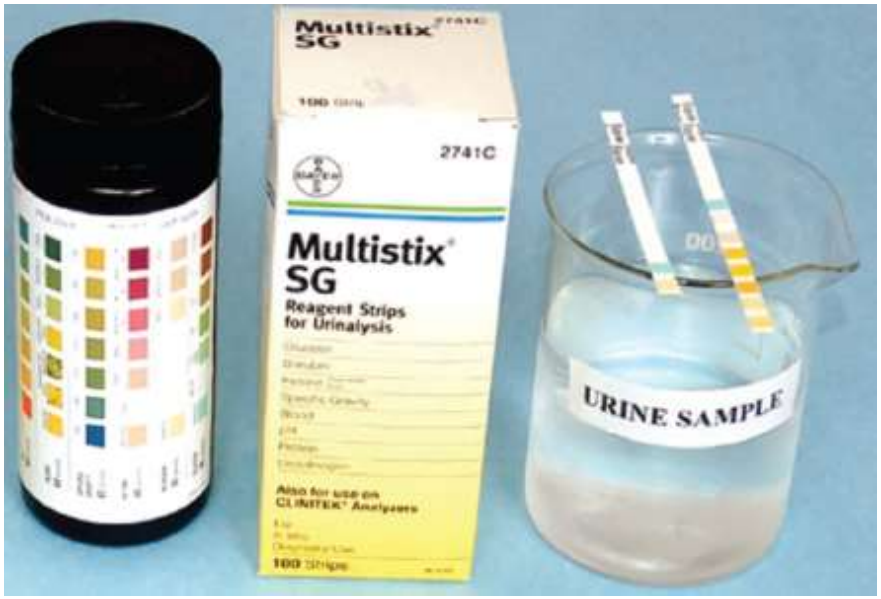
- *Appearance of white ring at the junction* indicates presence of protein.

CHEMICAL EXAMINATION
Test for Proteins

- **Reagent Strip Method**

Principle –

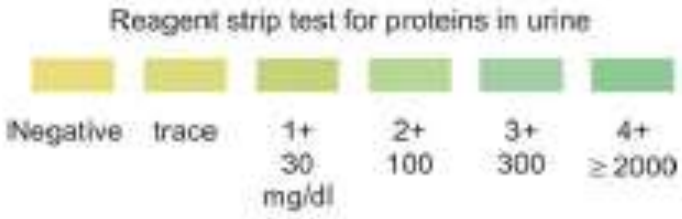
- The reagent area of the strip is coated with bromophenol blue indicator and buffered to an acid pH which changes color in the presence of proteins.



Method –

- Bromophenol coated strip is dipped in urine.
- Change in colour of strip indicates presence of proteins in urine and is compared with the colour chart provided for semiquantitative grading.

Interpretation -



Reagent strip test is mainly reactive to albumin.

It is false-negative in the presence of Bence Jones proteins, myoglobin, and hemoglobin.

CHEMICAL EXAMINATION

Microalbuminuria

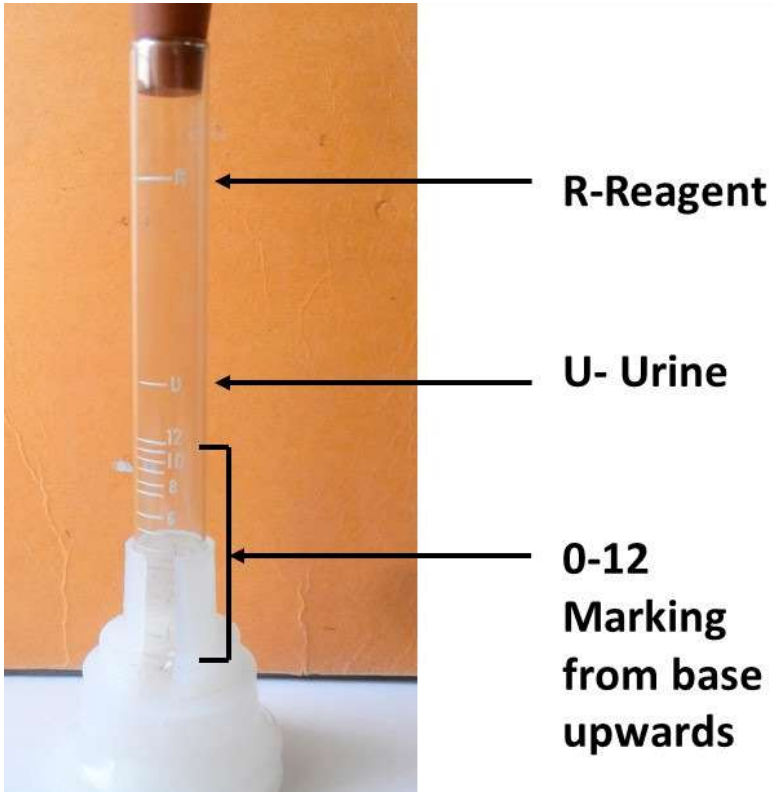
- Urinary excretion of 30 to 300 mg/24 hours (or 2-20 mg/dl) of albumin in urine.
- Significance –
  - earliest sign of renal damage in diabetes mellitus (diabetic nephropathy).
  - independent risk factor for cardiovascular disease in diabetes mellitus.
- Detection –
  - Measurement of albumin-creatinine ratio in a random urine sample.
  - Measurement of albumin in an early morning or random urine sample.
  - Measurement of albumin in a 24 hr sample .
  - Test strips that screen for microalbuminuria are available commercially.

CHEMICAL EXAMINATION

Quantitative Estimation of Proteins in Urine.

Esbach's albuminometer method

- Fill the albuminometer with urine up to mark U.
- Add Esbach's reagent (5g picric acid + 10g citric acid + 500ml of distilled water) up to mark R
- Stopper the tube, mix it and let it stand for 24 hours.
- Take the reading from the level of precipitation in the albuminometer tube and divide it by 10 to get the percentage of proteins.



CHEMICAL EXAMINATION

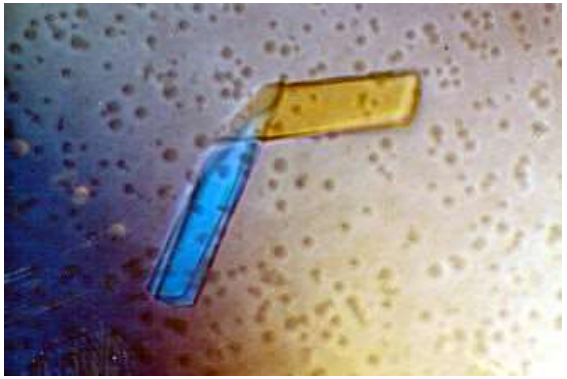
Quantitative Estimation of Proteins in Urine.

Turbidimetric method

- Take 1 ml of urine and 1 ml standard in two separate tubes.
- Add 4 ml of trichloroacetic acid to each tube.
- After 5 minutes take the reading with red filter (680 nm).

CHEMICAL EXAMINATION
Bence Jones Proteinuria

- **Bence Jones proteins** are monoclonal immunoglobulin light chains (either κ or λ) that are synthesized by neoplastic plasma cells.
- Excess production of these light chains occurs in plasma cell dyscrasias like multiple myeloma and primary amyloidosis.
- Because of their low molecular weight and high concentration they are excreted in urine (overflow proteinuria).



CHEMICAL EXAMINATION

Test for Bence Jones Proteinuria

- Upon **Δ**, **Bence Jones proteins** precipitate at temperatures between 40-60°C.

  (other proteins precipitate between 60-70°C)

- The precipitate disappears on further heating at 85-100°C.

  (while precipitate of other proteins does not)

- When **cooled** (60-85°C), there is reappearance of precipitate of Bence Jones proteins.

•This test is not specific for Bence Jones proteins and both false-positive and negative results can occur.
• This test has been replaced by protein electrophoresis of concentrated urine sample.
• Protein electrophoresis – Movement of charged particles through an electrolyte subjected to an electric field. e.g. M band in multiple myeloma

CHEMICAL EXAMINATION
Glucose

- Main indication for testing glucose in urine is detection of unsuspected diabetes mellitus or follow-up of known diabetic patients.
- All of the glucose filtered by the glomeruli is reabsorbed by the proximal renal tubules and returned to circulation.
- Normally a very small amount of glucose is excreted in urine that cannot be detected by the routine tests.

  (< 500 mg/24 hours or <15 mg/dl)
- Presence of detectable amounts of glucose in urine is **glycosuria**.
- Glycosuria results if the filtered glucose load exceeds the capacity of renal tubular reabsorption.

CHEMICAL EXAMINATION
Glucose

Causes of Glycosuria

Glycosuria with hyperglycemia:

- Endocrine diseases: diabetes mellitus, acromegaly, Cushing's syndrome, hyperthyroidism, pancreatic disease.
- Non-endocrine diseases: central nervous system diseases, liver disorders.
- Drugs: adrenocorticotrophic hormone, corticosteroids, thiazides.
- Alimentary glycosuria (Lag-storage glycosuria):
  - After a meal, there is rapid intestinal absorption of glucose leading to transient elevation of blood glucose above renal threshold.
  - This can occur in persons with gastrectomy or gastrojejunostomy and in hyperthyroidism.
  - Glucose tolerance test reveals a peak at 1 hour above renal threshold (which causes glycosuria); the fasting and 2-hour glucose values are normal.

CHEMICAL EXAMINATION
Glucose

Causes of Glycosuria

Glycosuria without hyperglycemia: Renal glycosuria

- This accounts for 5% of cases of glycosuria in general population.
- Renal threshold is the highest glucose level in blood at which glucose appears in urine and which is detectable by routine laboratory tests.
- The normal renal threshold for glucose is 180 mg/dl.
- The renal threshold is set below 180 mgs/dl but glucose tolerance is normal
- The disorder is transmitted as autosomal dominant.

CHEMICAL EXAMINATION
Test for Glucose

Benedict's qualitative test:

Composition of Benedict's qualitative reagent:

Copper sulphate 17.3 gram

Sodium carbonate 100 gram

Sodium citrate 173 gram

Distilled water 1000 ml

Principle –

- When urine is boiled in Benedict's qualitative solution, blue alkaline copper sulphate is reduced to red-brown cuprous oxide if abreducing agent is present.

CHEMICAL EXAMINATION
Test for Glucose

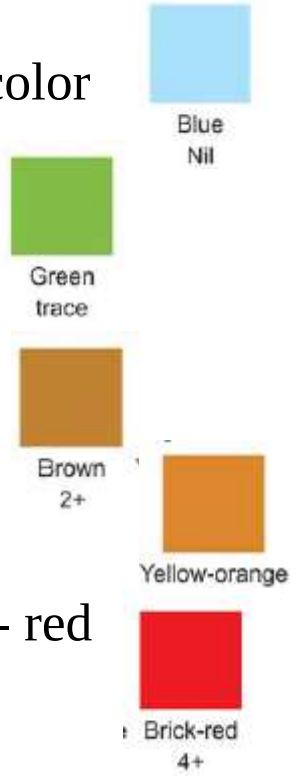
Benedict's qualitative test:

Methods:

- Add 0.5 ml (or 8 drops) of urine. Mix well.
- Boil over a flame for 2 minutes.
- Allow to cool at room temperature.
- Note the color change, if any.

Interpretation:

- The result is reported in grades as follows :
- Nil: no change from blue color
- Trace: Green without precipitate
- 1+ (approx. 0.5 grams/dl): Green with precipitate
- 2+ (approx. 1.0 grams/dl): Brown precipitate
- 3+ (approx. 1.5 grams/dl: Yellow-orange precipitate
- 4+ (> 2.0 grams/dl): Brick- red precipitate.



CHEMICAL EXAMINATION
Test for Glucose

Clinitest tablet method (Copper reduction tablet test):

- This is a modified form of Benedict's test in which the reagents are present in a tablet form.
- Sensitivity is 200 mgs/dl of glucose.

CHEMICAL EXAMINATION
Test for Glucose

Reagent strip method

- This test is **specific** for **glucose** and is therefore preferred over Benedict's and Clinitest methods.
- It is based on **glucose oxidase-peroxidase** reaction.
- Reagent area of the strips is impregnated with two enzymes - glucose oxidase and peroxidase and a chromogen.

$$\text{Glucose + Oxygen (room air)} \xrightarrow[\text{oxidase}]{\text{Glucose}} \text{Gluconic acid + Hydrogen peroxide}$$

$$\text{Hydrogen peroxide + Chromogen} \xrightarrow{\text{Peroxidase}} \text{Oxidized chromogen (Blue)} + H_2O$$

- Nature of chromogen and buffer system differ in different strips.
- The strip is dipped into the urine sample and color is observed after a specified time and compared with the color chart provided.
- This test is more sensitive than Benedict's qualitative test and specific only for glucose. Other reducing agents give negative reaction.

CHEMICAL EXAMINATION
Ketones

- Excretion of ketone bodies (**acetoacetic acid, β-hydroxybutyric acid**, and **acetone**) in urine is called as ketonuria.
- Ketones are breakdown products of fatty acids and their presence in urine is indicative of excessive fatty acid metabolism to provide energy.
- Normally ketone bodies are not detectable in the urine of healthy persons.
- If energy requirements cannot be met by metabolism of glucose (due to defective carbohydrate metabolism, low carbohydrate intake, or increased metabolic needs), then energy is derived from breakdown of fats →formation of ketone bodies

CHEMICAL EXAMINATION
Causes of Ketonuria

Decreased utilization of carbohydrates

- Uncontrolled diabetes mellitus with ketoacidosis →compensatory increased lipolysis → increase in the level of free fatty acids in plasma.
- Degradation of free fatty acids in the liver → the formation of acetoacetyl CoA which then forms ketone bodies.
- Ketone bodies are strong acids and produce H+ ions, which are neutralized by bicarbonate ions.
- Fall in bicarbonate (i.e. alkali) level produces ketoacidosis.
- Ketone bodies also increase the plasma osmolality and cause cellular dehydration.
- Presence of ketone bodies in urine may be a warning of impending ketoacidotic coma
- Glycogen storage disease (von Gierke's disease)

CHEMICAL EXAMINATION

Causes of Ketonuria

- Decreased availability of carbohydrates in the diet:

1. Starvation
2. Persistent vomiting in children
3. Weight reduction program (severe carbohydrate restriction with normal fat intake)

- Increased metabolic needs:
- a. Fever in children
- b. Severe thyrotoxicosis
- c. Pregnancy
- d. Protein calorie malnutrition

CHEMICAL EXAMINATION

Tests For Ketone Bodies

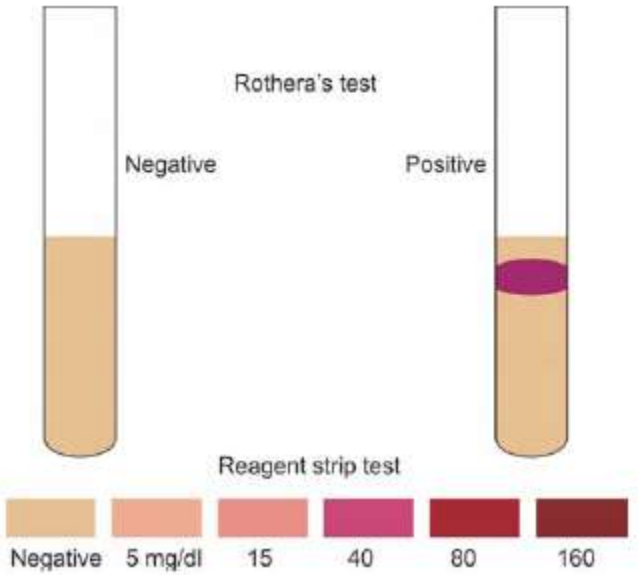
Rothera's Test

Principle :

Acetoacetic acid or acetone reacts with nitroprusside in alkaline solution to form a purple-colored complex.

Method:

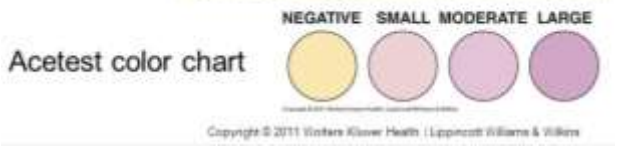
- Take 5 ml of urine in a test tube and saturate it with ammonium sulphate.
- Add a small crystal of sodium nitroprusside. Mix well.
- Slowly run along the side of the test tube liquor ammonia to form a layer.
- Immediate formation of a purple permanganate colored ring at the junction of the two fluids indicates a positive test



CHEMICAL EXAMINATION

Tests For Ketone Bodies

Acetest tablet test



This is Rothera's test in the form of a tablet.
The test is more sensitive than reagent strip test for ketones.
The Acetest tablet consists of sodium nitroprusside, glycine, and an alkaline buffer.
A purple lavender discoloration of the tablet indicates the presence of acetoacetate or acetone (≥ 5 mg/dl).
A rough estimate of the amount of ketone bodies can be obtained bybcomparison with the color chart provided by the manufacturer

CHEMICAL EXAMINATION
Tests For Ketone Bodies

Ferric chloride test (*Gerhardt's*)

- Addition of 10% ferric chloride solution to urine causes solution to become reddish or purplish if acetoacetic acid is present.
- The test is not specific since certain drugs (salicylate and L-dopa)give similar reaction.
- Sensitivity of the test is 25-50 mg/dl.

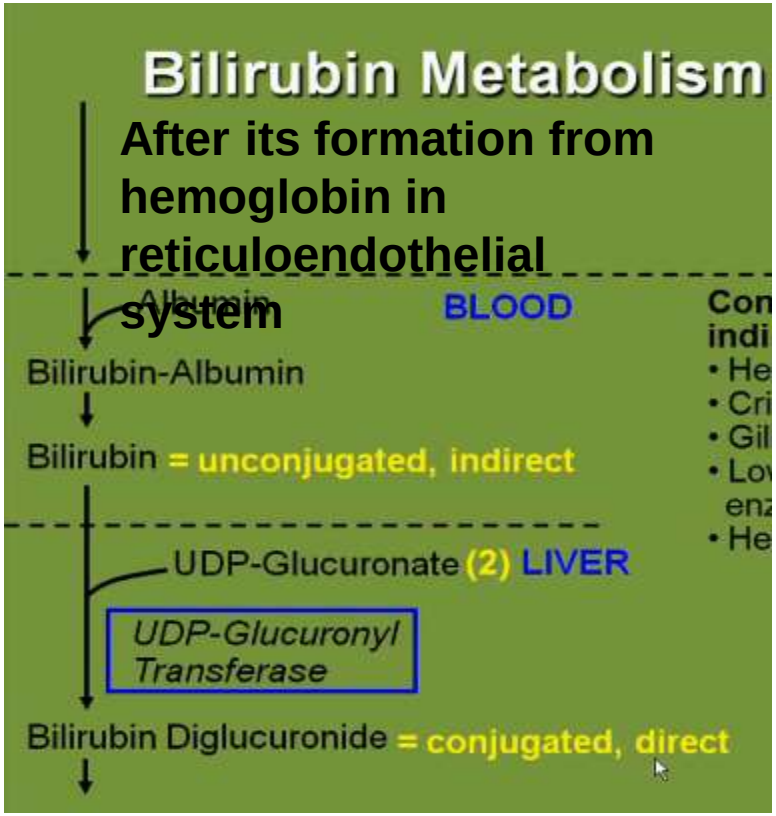
CHEMICAL EXAMINATION
Tests For Ketone Bodies

Reagent strip test

- *Reagent strips tests are modifications* of nitroprusside test.
- Their sensitivity is 5-10 mg/dl of acetoacetate.
- If exposed to moisture, reagent strips often give false-negative result.
- Ketone pad on the strip test is especially vulnerable to improper storage and easily gets damaged.

CHEMICAL EXAMINATION
Bile Pigment (Bilirubin)

- Bilirubin (a breakdown product of hemoglobin) is undetectable in the urine of normal persons.
- Presence of bilirubin in urine is called as bilirubinuria.



Unconjugated bilirubin is not water-soluble, is bound to albumin, and cannot pass through the glomeruli. Therefore, **it does not appear in urine**

Conjugated bilirubin is **water soluble**, is filtered by the glomeruli, and therefore **appears in urine**.

CHEMICAL EXAMINATION

Bile Pigment (Bilirubin)

Detection of bilirubin in urine (along with urobilinogen) is helpful in the differential diagnosis of jaundice

Urine test	Hemolytic Jaundice	Hepatocellular Jaundice	Obstructive Jaundice
Bilirubin	Absent	Present	Present
Urobilinogen	Increased	Increased	Absent

•In acute viral hepatitis, bilirubin appears in urine even before jaundice is clinically apparent.

•Presence of bilirubin in urine indicates conjugated hyperbilirubinemia (obstructive or hepatocellular jaundice).

CHEMICAL EXAMINATION

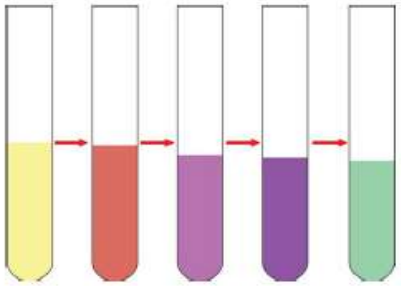
Tests For Detection of Bilirubin in Urine

1. Foam test:

- About 5 ml of urine in a test tube is shaken and observed for development of yellowish foam.
- Similar result is also obtained with proteins and highly concentrated urine.
- In normal urine, foam is white.

2. Gmelin's test:

- Take 3 ml of concentrated nitric acid in a test tube and slowly place equal quantity of urine over it.
- The tube is shaken gently, play of colors (yellow, red, violet, blue, & green) indicates +ve test.



3. Lugol iodine test:

Take 4 ml of Lugol iodine solution (Iodine 1 gm, potassium iodide 2 gm, and distilled water to make 100 ml) in a test tube and add 4 drops of urine. Mix by shaking. Development of green color indicates positive test.

CHEMICAL EXAMINATION

Tests For Detection of Bilirubin in Urine

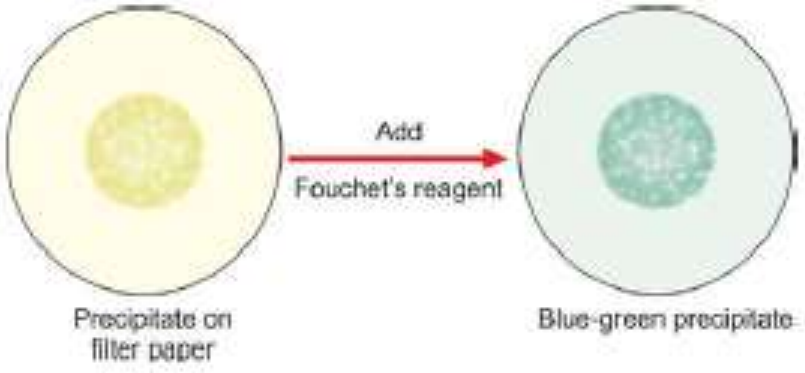
4. **Fouchet's test**: A simple and sensitive test.

i. Add **2.5 ml of 10% of barium chloride** to **5 ml of fresh urine** in a test tube and mix well. A precipitate of sulphates appears to which bilirubin is bound (barium sulphate-bilirubin complex).

ii. Filter to obtain the precipitate on a filter paper.

iii. To the precipitate on the filter paper, add 1 drop **of Fouchet's reagent** (25 g of trichloroacetic acid, 10 ml of 10% ferric chloride, and distilled water 100 ml).

iv. Immediate development of blue-green color around the drop indicates presence of bilirubin.



CHEMICAL EXAMINATION

Tests For Detection of Bilirubin in Urine

5. Reagent strips or tablets impregnated with diazo reagent:

- These tests are based on reaction of bilirubin with diazo reagent.
- The color change is proportional to the concentration of bilirubin.
- Tablets (Ictotest) detect 0.05-0.1 mg of bilirubin/dl of urine.
- The reagent strip tests are less sensitive (0.5 mg/dl).

CHEMICAL EXAMINATION
Bile Salts

- Bile salts are salts of bile acids: cholic, deoxycholic, chenodeoxycholic, and lithocholic.
- These bile acids combine with glycine or taurine to form complex salts or acids.
- Bile salts enter the small intestine through the bile and act as detergents to emulsify fat and reduce the surface tension on fat droplets so that enzymes (lipases) can breakdown the fat.
- In the terminal ileum, bile salts are absorbed and enter in the blood stream from where they are taken up by the liver and re-excreted in bile (enterohepatic circulation).

CHEMICAL EXAMINATION

Bile Salts

- Bile salts along with bilirubin can be detected in urine in cases of obstructive jaundice.
- Bile salts and conjugated bilirubin regurgitate into blood from biliary canaliculi (due to increased intrabiliary pressure) and are excreted in urine.
- The test used for the detection of bile salts is Hay's surface tension test.
- The property of bile salts to lower the surface tension is utilized in this test.

CHEMICAL EXAMINATION
Tests For Bile Salts

Hay's surface tension test :

- Take some fresh urine in a conical glass tube.
- Urine should be at the room temperature.
- Sprinkle on the surface particles of sulphur.
- If bile salts are present, sulphur particles sink to the bottom because of lowering of surface tension by bile salts.
- If sulphur particles remain on the surface of urine, bile salts are absent.
- Thymol (used as a preservative) gives false positive test.

CHEMICAL EXAMINATION

Urobilinogen

- Conjugated bilirubin excreted into the duodenum through bile is converted by bacterial action to urobilinogen in the intestine.
- Major part is eliminated in the feces.
- A portion of urobilinogen is absorbed in blood, which
- undergoes recycling (enterohepatic circulation)
- A small amount, which is not taken up by the liver, is excreted in urine.
- Urobilinogen is colorless; upon oxidation it is converted to urobilin, which is orange-yellow in color.
- Normally about 0.5-4 mg of urobilinogen is excreted in urine in 24 hours.
- Urinary excretion of urobilinogen shows diurnal variation with highest levels in afternoon.
- A 2-hour post-meal sample is usually preferred.

CHEMICAL EXAMINATION

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CHEMICAL EXAMINATION
Urobilinogen

Causes of Increased Urobilinogen in Urine

1. **Hemolysis**
   - Increased urobilinogen in urine without bilirubin
   - Excessive destruction of red cells leads to hyperbilirubinemia and therefore increased formation of urobilinogen in the gut.
2. **Hemorrhage in tissues:**
   - There is increased formation of bilirubin from destruction of red cells.

Causes of Reduced Urobilinogen in Urine

1. **Obstructive jaundice:**

In biliary tract obstruction, delivery of bilirubin to the intestine is restricted and very little or no urobilinogen is formed → clay-colored stools.

2. **Reduction of intestinal bacterial flora:**

This prevents conversion of bilirubin to urobilinogen in the intestine.
It is observed in neonates and following antibiotic treatment

CHEMICAL EXAMINATION
Tests For **Urobilinogen**

Ehrlich's aldehyde test

- Ehrlich's reagent (*p-dimethylaminobenzaldehyde)* reacts with urobilinogen in urine to produce a pink color.
- Intensity of color developed depends on the amount of urobilinogen present.
- Take 5 ml of fresh urine in a test tube.
- Add 0.5 ml of Ehrlich's aldehyde reagent (which consists of ydrochloric acid 20 ml, distilled water 80 ml, and paradimethylaminobenzaldehyde 2 gm).
- Allow to stand at room temperature for 5 minutes.
- **Development of pink color indicates normal amount of urobilinogen.**
- **Dark red color means increased amount of urobilinogen.**

CHEMICAL EXAMINATION
Tests For **Urobilinogen**

Watson-Schwartz Test

- Distinguish between both urobilinogen and porphobilinogen.
- Add 1-2 ml of chloroform, shake for 2 minutes and allow to stand.
- Pink color in the chloroform layer indicates presence of urobilinogen, while pink coloration of aqueous portion indicates presence of porphobilinogen.

- False-negative reaction can occur in the presence of

(i) urinary tract infection (nitrites oxidize urobilinogen to urobilin)

(ii) antibiotic therapy (gut bacteria which produce urobilinogen are destroyed).

CHEMICAL EXAMINATION
Tests For **Urobilinogen**

Reagent strip method:

- This method is specific for urobilinogen.
- Test area is impregnated with either *p-dimethylaminobenzaldehyde or 4-methoxybenzene* diazonium tetrafluoroborate.

CHEMICAL EXAMINATION
Blood

- The presence of abnormal number of intact red blood cells in urine is called as hematuria.

Causes of Hematuria

1. Diseases of urinary tract

- **Glomerular diseases:** Glomerulonephritis, Berger'sdisease, lupus nephritis, Henoch-S.chonlein purpura.
- **Non-glomerular diseases**: Calculus, tumor, infection, tuberculosis, pyelonephritis, hydronephrosis, polycystic kidney disease, trauma, after strenuous physical exercise, diseases of prostate (benign hyperplasia of prostate, carcinoma of prostate.

2. ***Hematological conditions:***

- *Coagulation disorders, sickle* cell disease

CHEMICAL EXAMINATION

Tests for Detection of Blood in Urine

1. **Microscopic examination of urinary sediment:**

- Definition of microscopic hematuria is presence of 3 or more number of red blood cells per high power field on microscopic examination of urinary sediment in two out of three properly collected samples.

2. **Chemical tests:**

- **Benzidine test:**
  - Make saturated solution of benzidine in glacial acetic acid.
  - Mix 1 ml of this solution with 1 ml of hydrogen peroxide in a test tube.
  - Add 2 ml of urine.
  - If green or blue color develops within 5 minutes, the test is positive.
- **Orthotoluidine test:**
  - In this test, instead of benzidine, orthotoluidine is used.
  - It is more sensitive than benzidine test.
- **Reagent strip test:**
  - Various reagent strips are commercially available which use different chromogens (o-toluidine, tetramethylbenzidine).

CHEMICAL EXAMINATION

Tests for Detection of Blood in Urine

Chemical tests are positive in hematuria, hemoglobinuria and myoglobinuria.

Parameter	*Hematuria*	*Hemoglobinuria*	*Myoglobinuria*
1. Urine color	Normal, smoky, red, or brown	Pink, red, or brown	Red or brown
2. Plasma color	Normal	Pink	Normal
3. Urine test based on peroxidase activity	Positive	Positive	Positive
4. Urine microscopy	Many red cells	Occasional red cell	Occasional red cell
5. Serum haptoglobin	Normal	Low	Normal
6. Serum creatine kinase	Normal	Normal	Markedly increased

CHEMICAL EXAMINATION
Chemical Tests for Significant Bacteriuria

1. **Nitrite test:**

- **Nitrites are not present in normal urine.**
- Ingested nitrites are converted to nitrate and excreted in urine.
- If gram-negative bacteria (e.g. *E.coli,Salmonella, Proteus, Klebsiella, etc.) are present in urine*
- Nitrites are then detected in urine by reagent strip tests.

2. **Leucocyte esterase test:**

- **It detects esterase enzyme** released in urine from granules of leucocytes.
- Thus the test is positive in pyuria. If this test is positive, urine culture should be done.
- The test is not sensitive to leucocytes < 5/HPF.

URINE EXAMINATION

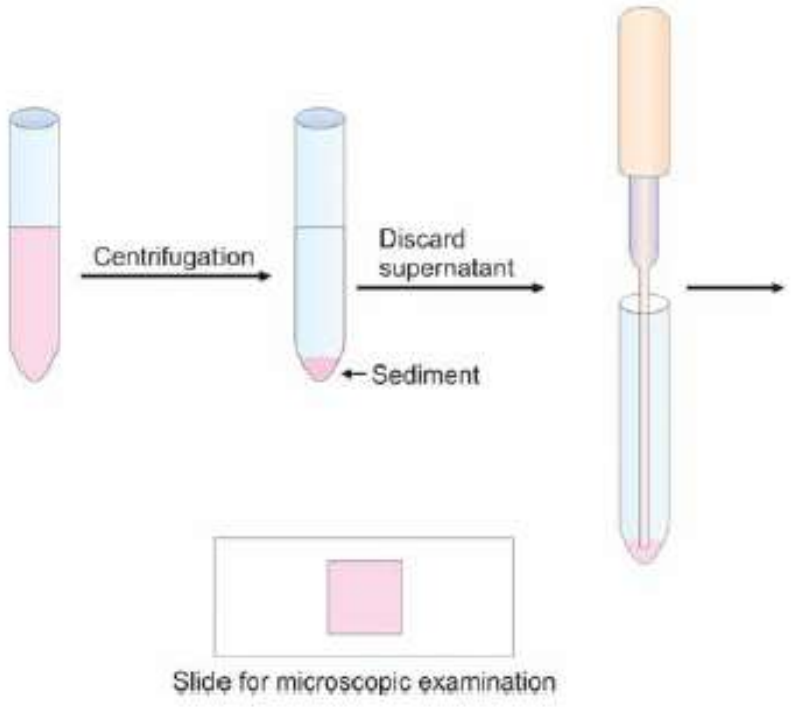
- **PHYSICAL EXAMINATION**
- **CHEMICAL EXAMINATION**
- **MICROSCOPIC EXAMINATION**

MICROSCOPIC EXAMINATION

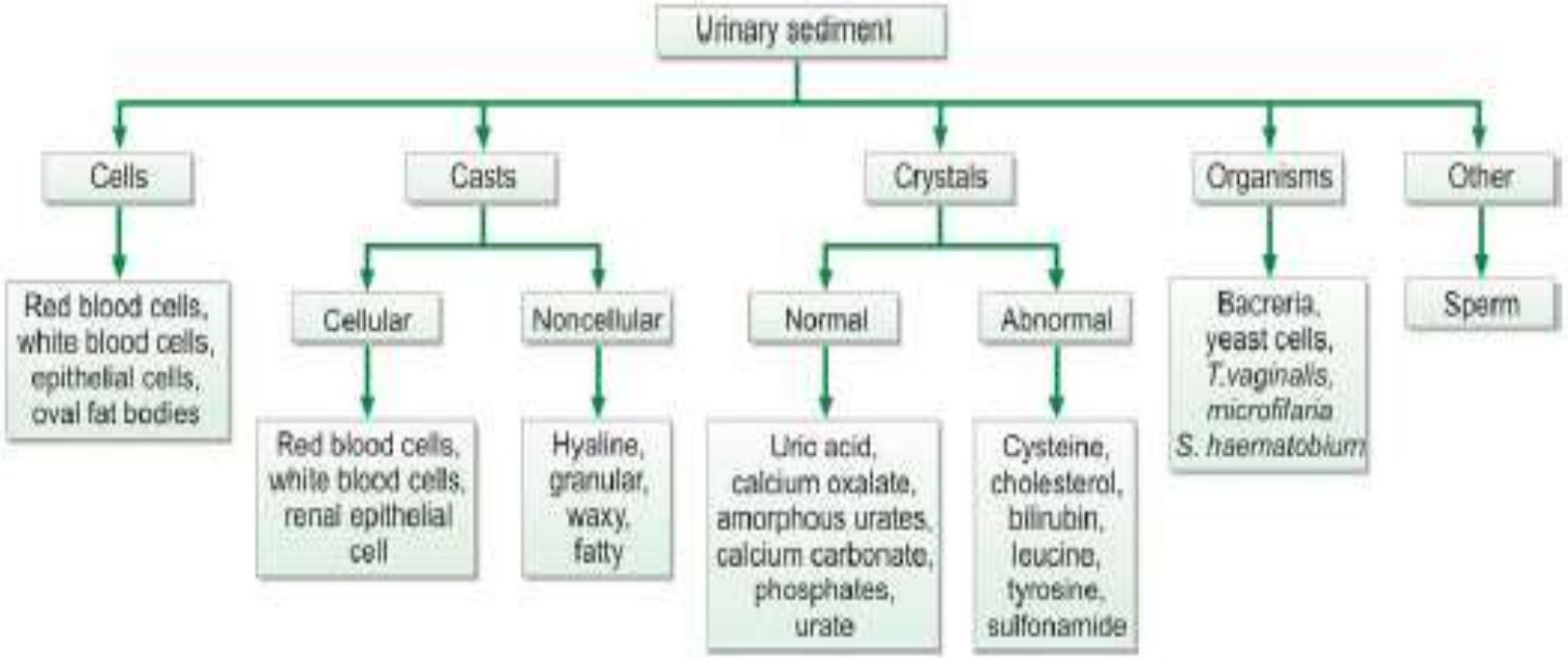
- Normal urine microscopy contains few epithelial cells, occasional RBC's, few crystals.
- Urine consists of various microscopic, insoluble, solid elements in suspension.
- These elements are classified as organized or unorganized.
- **Organized substances** include red blood cells, white blood cells, epithelial cells, casts, bacteria, and parasites.
- **Unorganized substances** are crystalline and amorphous material which are suspended in urine and on standing they settle down and sediment at the bottom of the container→**urinary deposits or urinary sediments.** The cellular elements are best preserved in acid, hypertonic urine; they deteriorate rapidly **in** alkaline, hypotonic solution.

MICROSCOPIC EXAMINATION

- Microscopic urinalysis is done by pouring the urine sample into a test tube and centrifuging it for a few minutes.
- The top liquid part (the supernatant) is discarded.
- The solid part left in the bottom of the test tube (the urine sediment) is mixed with the remaining drop of urine in the test tube
- one drop of this is analyzed under a microscope



MICROSCOPIC EXAMINATION

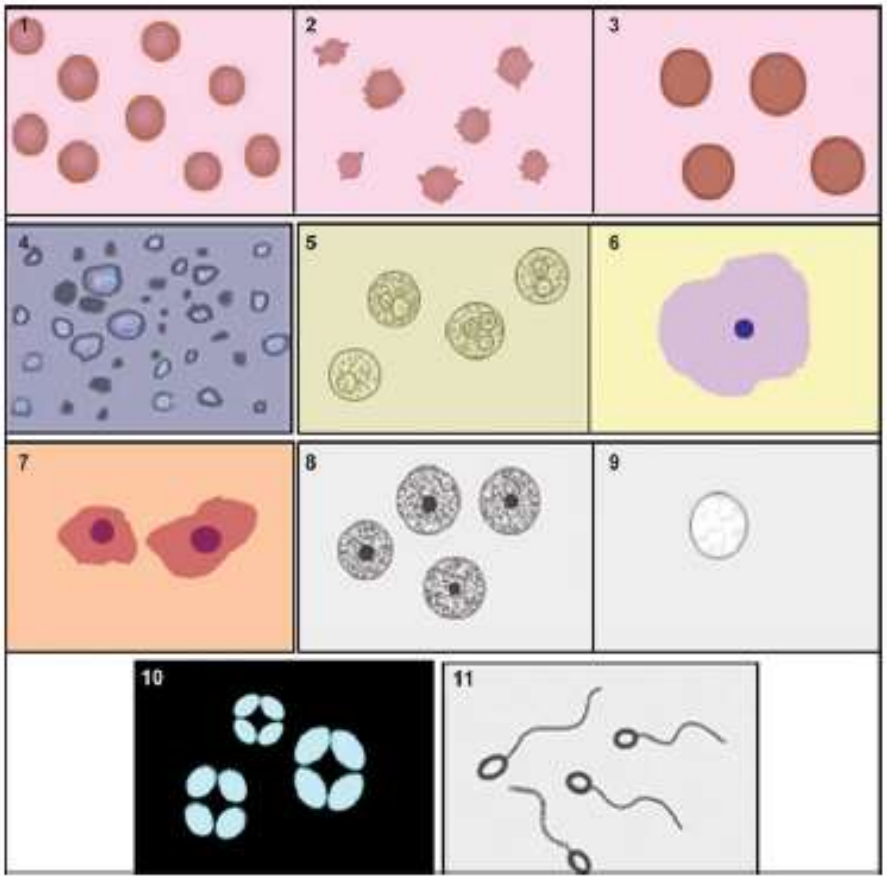


MICROSCOPIC EXAMINATION
Urinary findings in renal diseases

Condition	Albumin	RBCs/HPF	WBCs/HPF	Casts/LPF	Others
1. Normal	0-trace	0-2	0-2	Occasional (Hyaline)	–
2. Acute glomerulonephritis	1-2+	Numerous; dysmorphic	0-few	Red cell, granular	Smoky urine or hematuria
3. Nephrotic syndrome	>4+	0-few	0-few	Fatty, hyaline, Waxy, epithelial	Oval fat bodies, lipiduria
4. Acute pyelonephritis	0-1+	0-few	Numerous	WBC, granular	WBC clumps, bacteria, nitrite test

HPF: High power field; LPF: Low power field; RBCs: Red blood cells; WBCs: White blood cells.

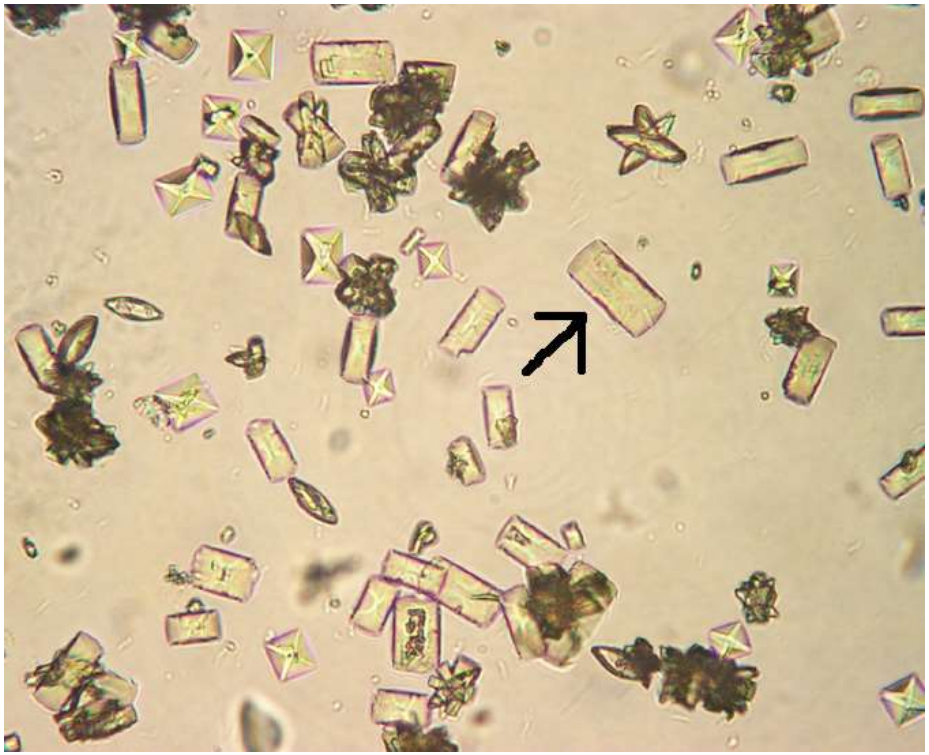
MICROSCOPIC EXAMINATION
Cells in urine



1.24: Cells in urine (1) Isomorphic red blood cells, (2) Crenated red cells, (3) Swollen red cells, (4) Dysmorphic red cells, (5) White blood cells (pus cells), (6) Squamous epithelial cell, (7) Transitional epithelial cells, (8) Renal tubular epithelial cells, (9) Oval fat bodies, (10) Maltese cross pattern of oval fat bodies, and (11) spermatozoa

MICROSCOPIC EXAMINATION
Crystals

- Crystals are refractile structures with a definite geometric shape due to orderly 3-dimensional arrangement of its atoms and molecules.
- Amorphous material (or deposit) has no definite shape and is commonly seen in the form of granular aggregates or clumps



MICROSCOPIC EXAMINATION

Crystals

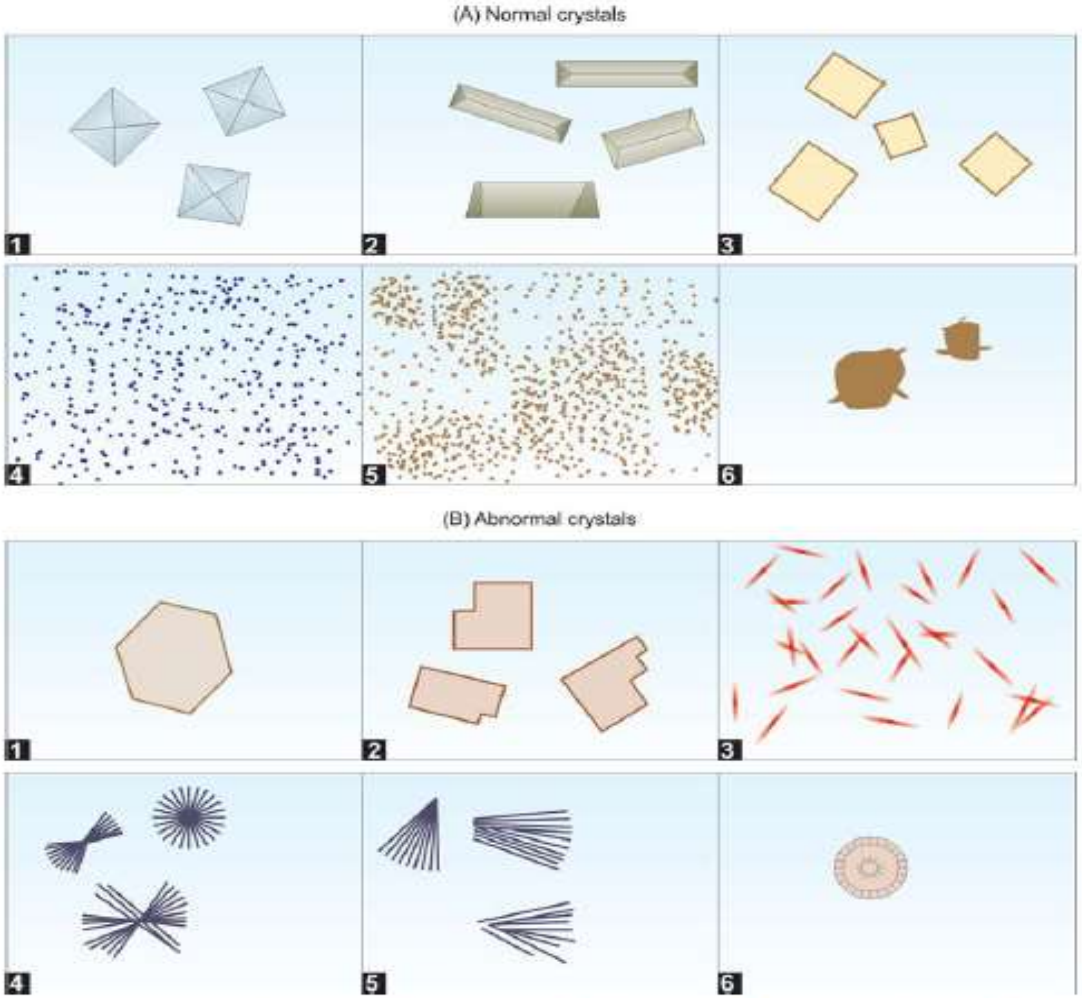


Fig. 1.28: Crystals in urine. (A) Normal crystals: (1) Calcium oxalate, (2) Triple phosphates, (3) Uric acid, (4) Amorphous phosphates, (5) Amorphous urates, (6) Ammonium urate. (B) Abnormal crystals: (1) Cysteine, (2) Cholesterol, (3) Bilirubin, (4) Tyrosine, (5) Sulfonamide, and (6) Leucine

MICROSCOPIC EXAMINATION

Crystals

Crystals in acidic urine

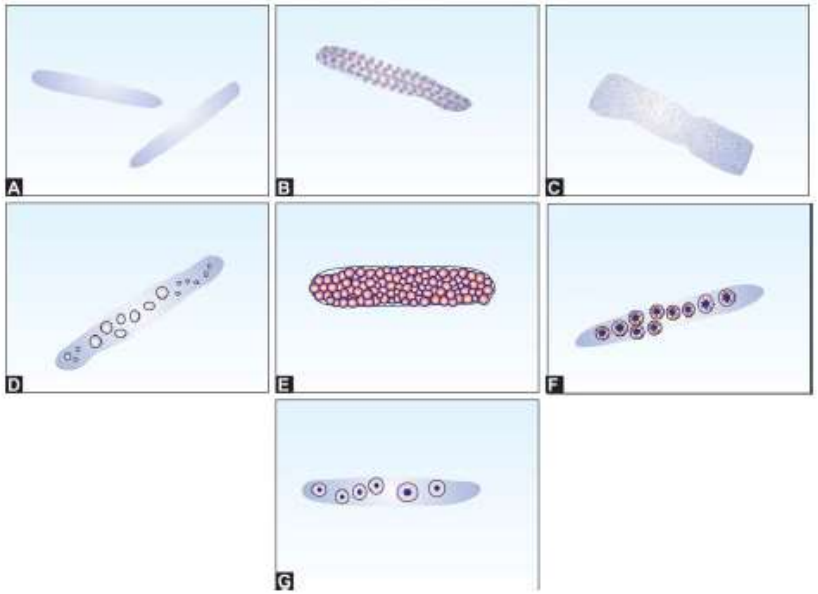
- Uric acid
- Calcium oxalate
- Cystine
- Leucine

Crystals in alkaline urine

- Ammonium magnesium phosphates(triple phosphate crystals)
- Calcium carbonate
- Amorphous phosphates
- Ammonium urate crystals

MICROSCOPIC EXAMINATION
Casts in urine

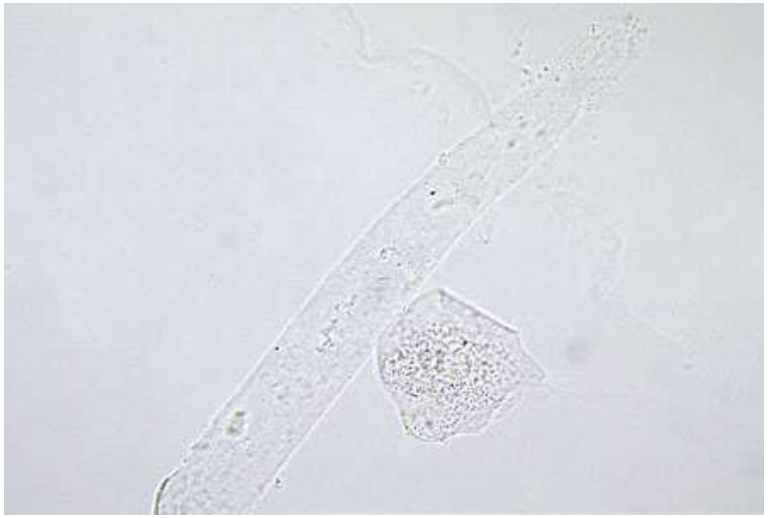
- **Urinary casts** are cylindrical aggregations of particles that form in the distal nephron, dislodge and pass in the urine.
- Casts are of two main types:
  - **Noncellular:** Hyaline, granular, waxy, fatty
  - **Cellular:** Red blood cell, white blood cell, renal tubular epithelial cell.



Jrinary casts: (A) Hyaline cast, (B) Granular cast, (C) Waxy cast, (D) Fatty cast, (E) Red cell cast, (F) White cell cast, and (G) Epithelial cast

MICROSCOPIC EXAMINATION
Casts in urine
Hyaline casts

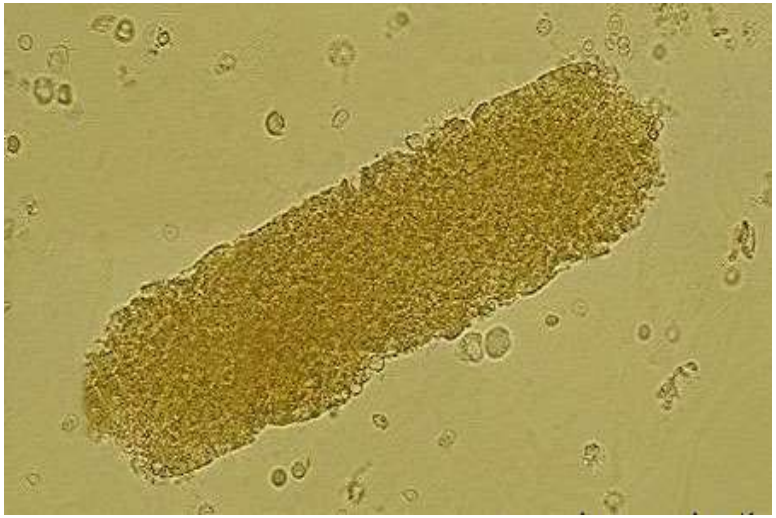
- Most common type of casts which are composed of solidified Tamm-Horsfall mucoprotein.
- They have smooth texture and a refractive index very close to that of the surrounding fluid.



They may be seen in healthy patients, increased numbers during dehydration, exercise or diuretic medicines.

MICROSCOPIC EXAMINATION
Casts in urine
Granular Casts

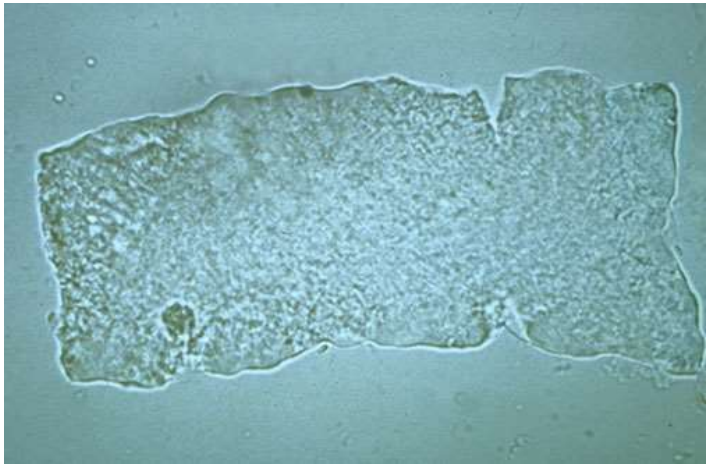
- Granular casts result either from the degeneration of cellular casts, or direct aggregation of plasma proteins or immunoglobulin light chains.
- They have a textured appearance which ranges from fine to coarse in character.



They are seen after sternous exercise, chronic renal diseases, acute tubular necrosis etc.

MICROSCOPIC EXAMINATION
Casts in urine
Waxy Casts

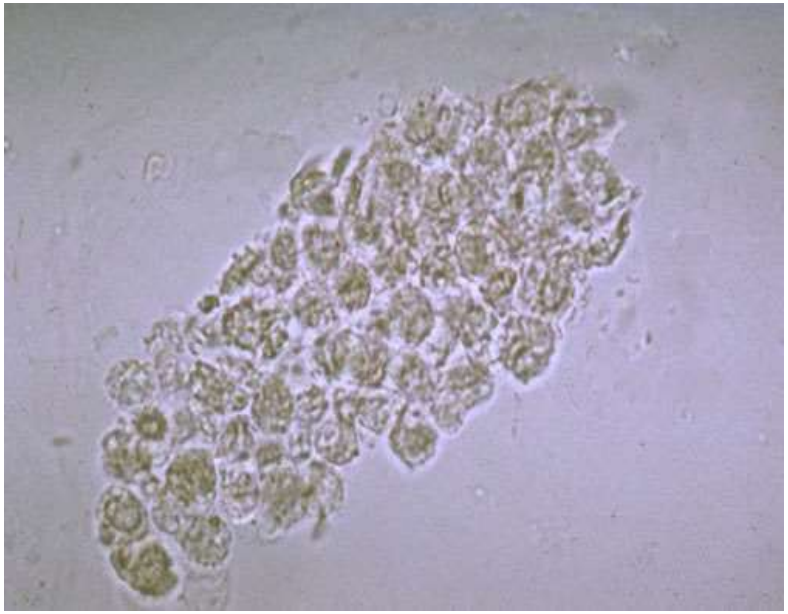
- Waxy casts represent the final stage of degeneration of cellular casts. They are more refractile



seen in tubular injury of a more chronic nature than granular or cellular casts like **severe chronic renal disease** and **renal amyloidosis**. These casts are also called ***renal failure casts***.

MICROSCOPIC EXAMINATION
Casts in urine
Fatty Casts

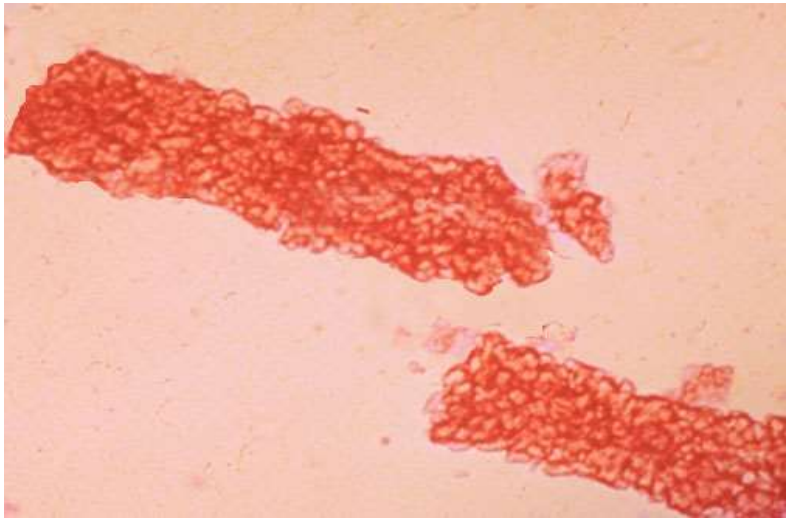
- Fatty casts are formed by the breakdown of lipid-rich epithelial cells. These contain lipid droplets within the protein matrix of the cast and are identified by the presence of refractile lipid droplets.
-



They are usually seen in the conditions like tubular degeneration, nephrotic syndrome, hypothyroidism etc.

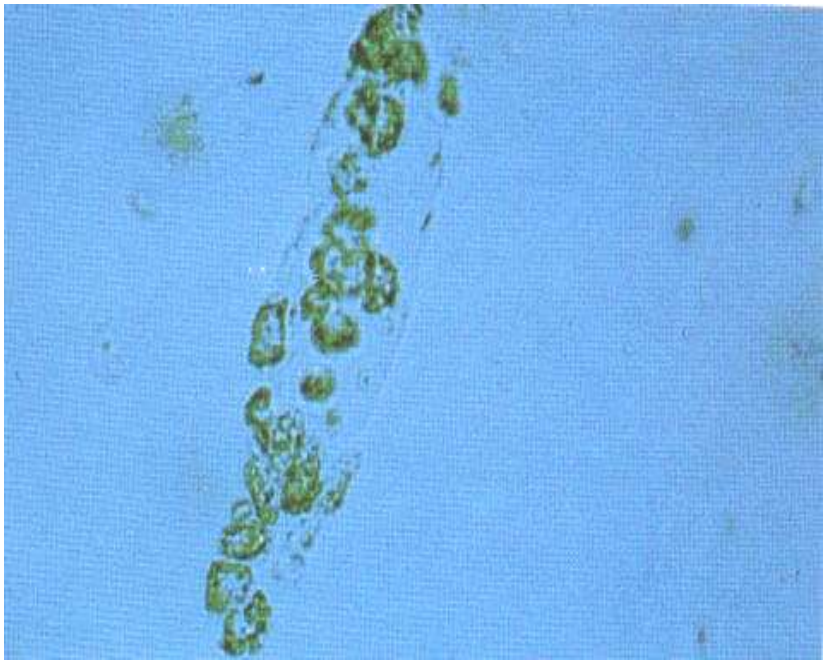
MICROSCOPIC EXAMINATION
Casts in urine
Red blood cell casts

- The presence of red blood cells within the cast is always pathologic, and is strongly indicative of ***glomerular damage.***
- They are usually associated with nephritic syndromes.



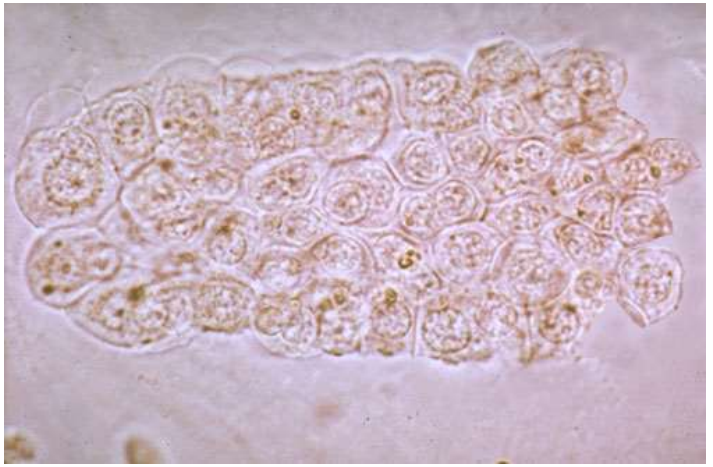
MICROSCOPIC EXAMINATION
Casts in urine
White blood cell casts

- White blood cells (generally neutrophils) are present within or upon casts.
- Indicative of inflammation or infection.
- These casts are typical for acute pyelonephritis



MICROSCOPIC EXAMINATION
Casts in urine
Renal Tubular Epithelial Cell Casts

- These casts are composed of renal epithelial cells.
- These casts are seen in conditions such as renal tubular necrosis, viral disease (such as CMV nephritis), and kidney transplant rejection



MICROSCOPIC EXAMINATION
Organisms in urine

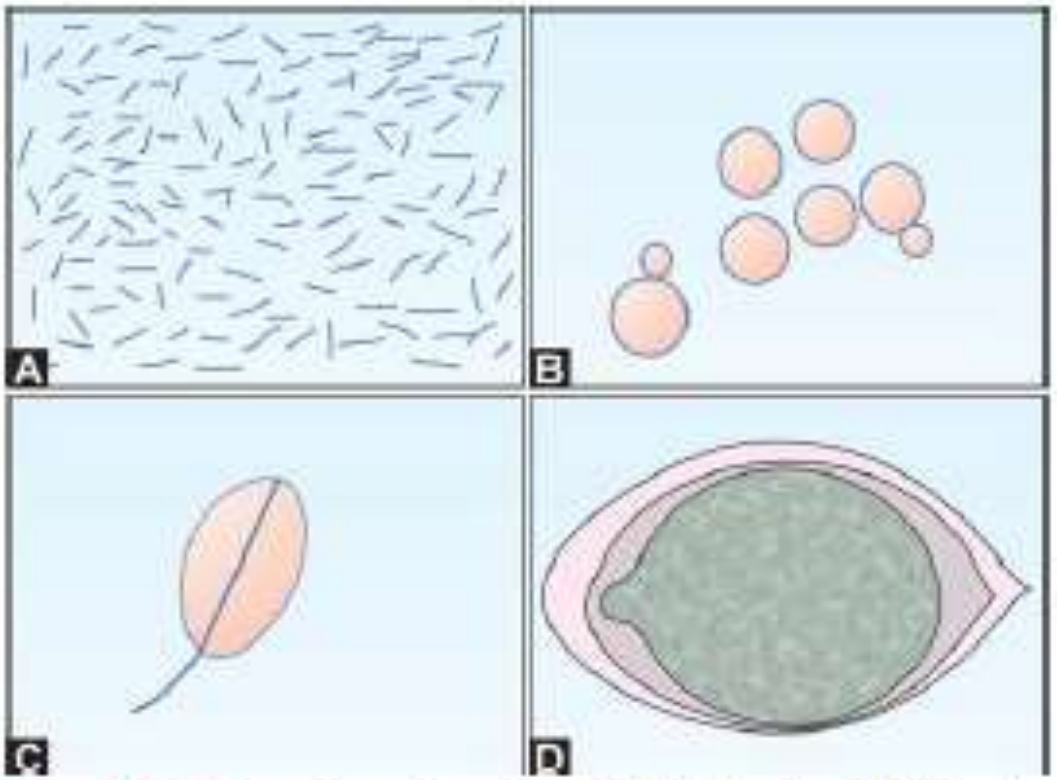


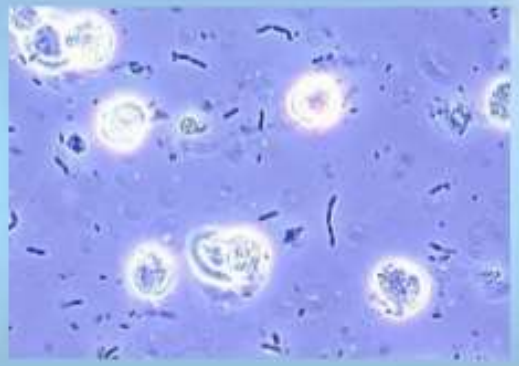
Fig. 1.25: Organisms in urine: (A) Bacteria, (B) Yeasts, (C) *Trichomonas*, and (D) Egg of *Schistosoma haematobium*

MICROSCOPIC EXAMINATION
Organisms in urine

S. haematobium

Budding yeast

Trichomonas vaginalis



bacteria and many white cells

Transitional Epi cells

Renal epithelial cells


Thank you
for Listening!