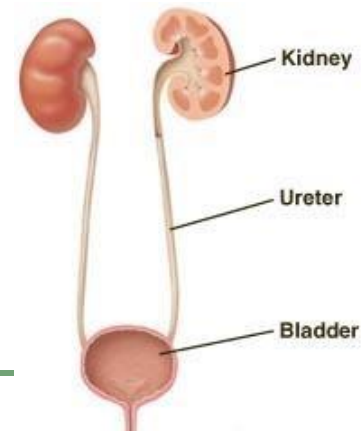


Urine analysis for it's Normal & Abnormal Constituents



Urinary System

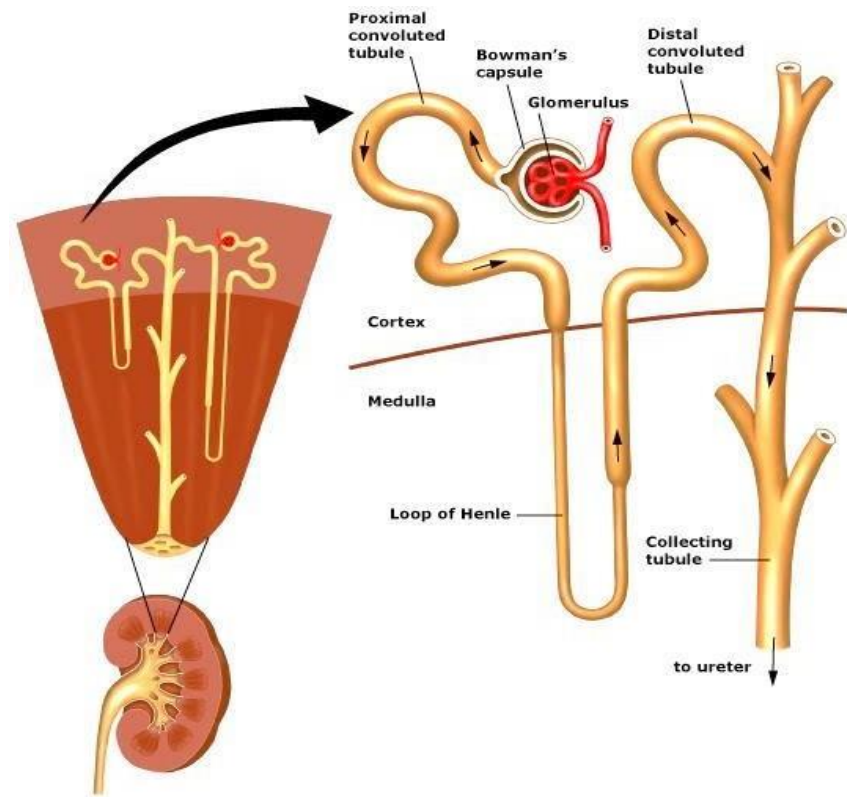
- The kidneys remove waste product from the blood through small filtering units called **nephrons**.
- Each nephron consists of a ball of small blood capillaries, called a glomerulus, and a small tube called a renal tubule.
- The **kidneys** form urine, which passes through the **ureters** to the **bladder** for storage prior to excretion.
- - **Waste product** of protein metabolism are excreted,
- **electrolyte levels** are controlled
- and **pH** (acid-base balance) is maintained by excretion of H⁺ ions.



Urine Formation :

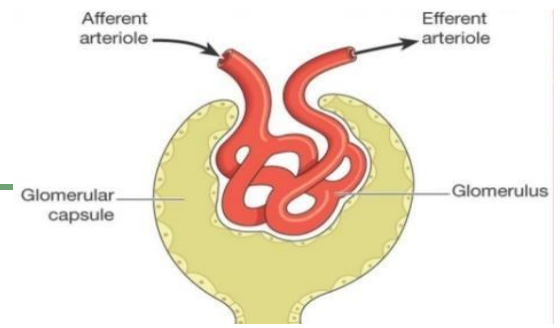
There are three processes involved in the formation of urine:

- Filtration .
- Tubular reabsorption.
- Tubular secretion.



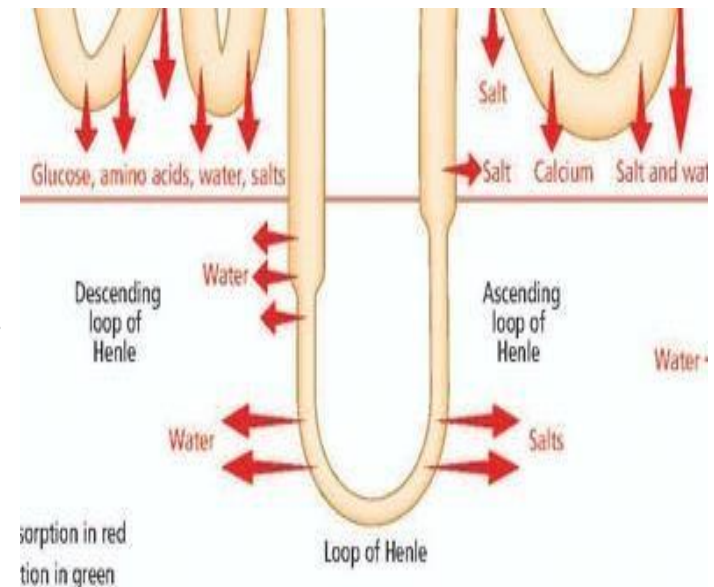
1- Filtration:

- This takes place through the **semipermeable** wall of glomerulus and glomerular capsule .
- Water and **small** molecules move from the glomerulus to the inside of the glomerular capsule.
- Molecules which have molecular weight **more** than 70,000 Dalton **can not** pass the glomerulus.
- Blood cells, plasma proteins and other large molecules are **too large** to filtrate.
- Inside the glomerular capsule now contains **glomerular filtrate** which is very similar in composition of plasma except of **plasma proteins** and **blood cells**.
- (non-selective filtration occurs).



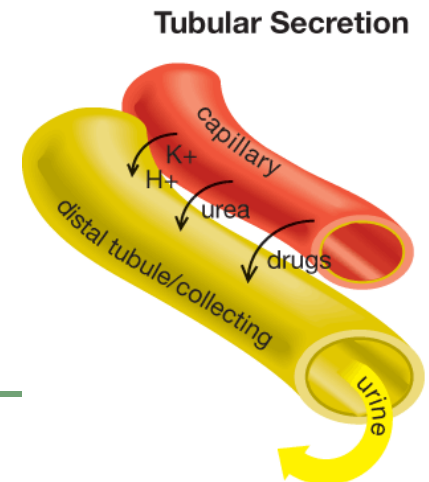
2- Tubular Reabsorption:

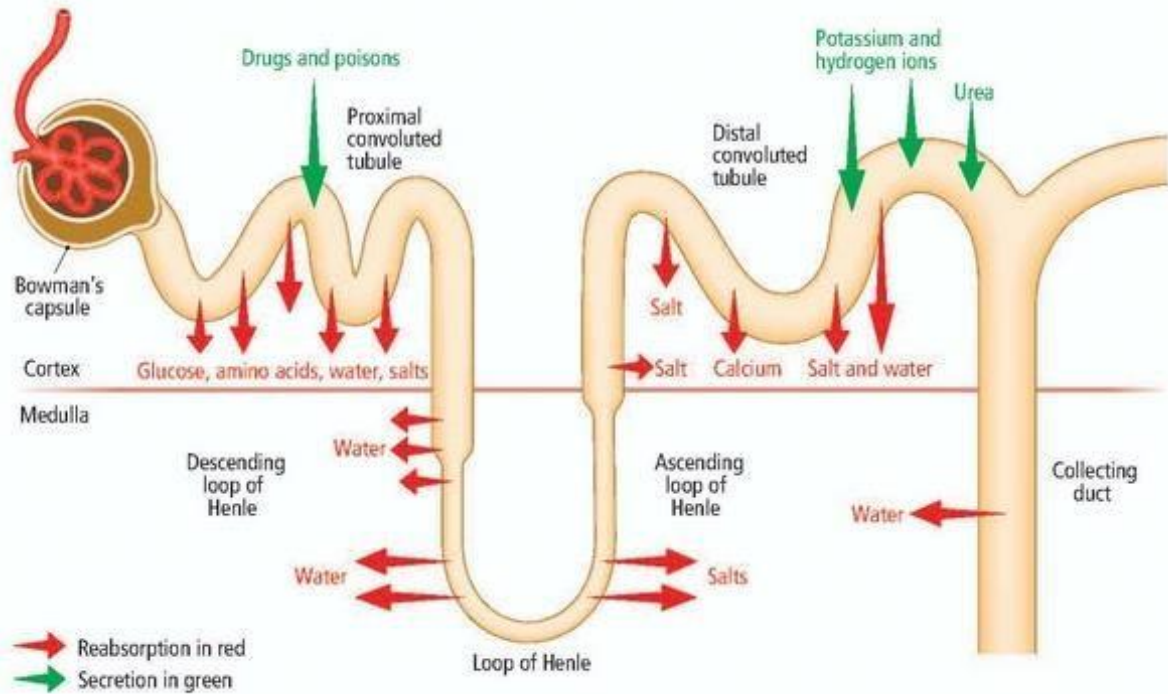
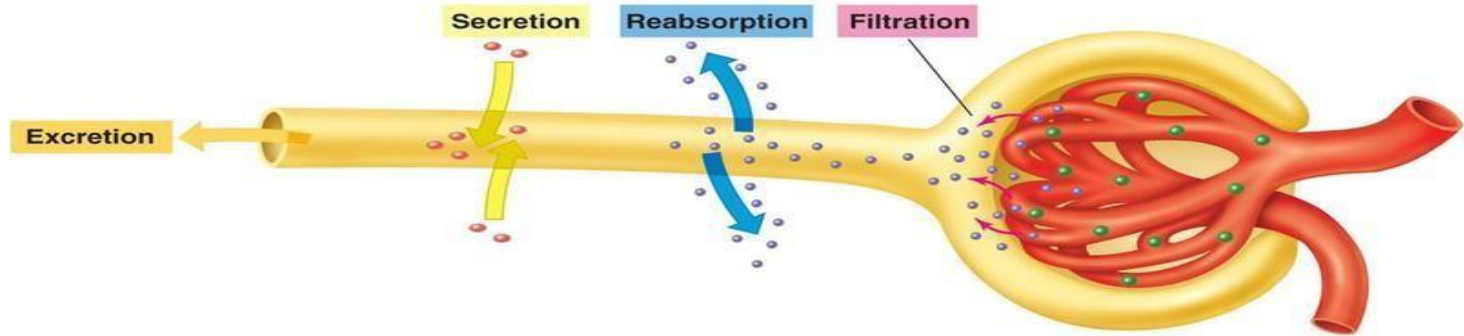
- Reabsorption is the movement of water and solutes from the tubule **back into the blood**.
- As molecules and ions are passively and actively reabsorbed from the nephron into the blood of the peritubular capillary network.
- Nutrients such as glucose and amino acids return to the peritubular capillaries almost exclusively at the proximal convoluted tubule.
- every substance has a **maximum rate of transport**.



3- Tubular Secretion:

- Is a second way by which substances are removed from blood and added to the tubular fluid.
- Hydrogen ions (H⁺), creatinine, and drugs such as penicillin are some of the substances moved by **active transport** from blood into the kidney tubule.
- is a process in which the renal tubule extracts chemicals from the capillary blood and secretes them into the tubular fluid.





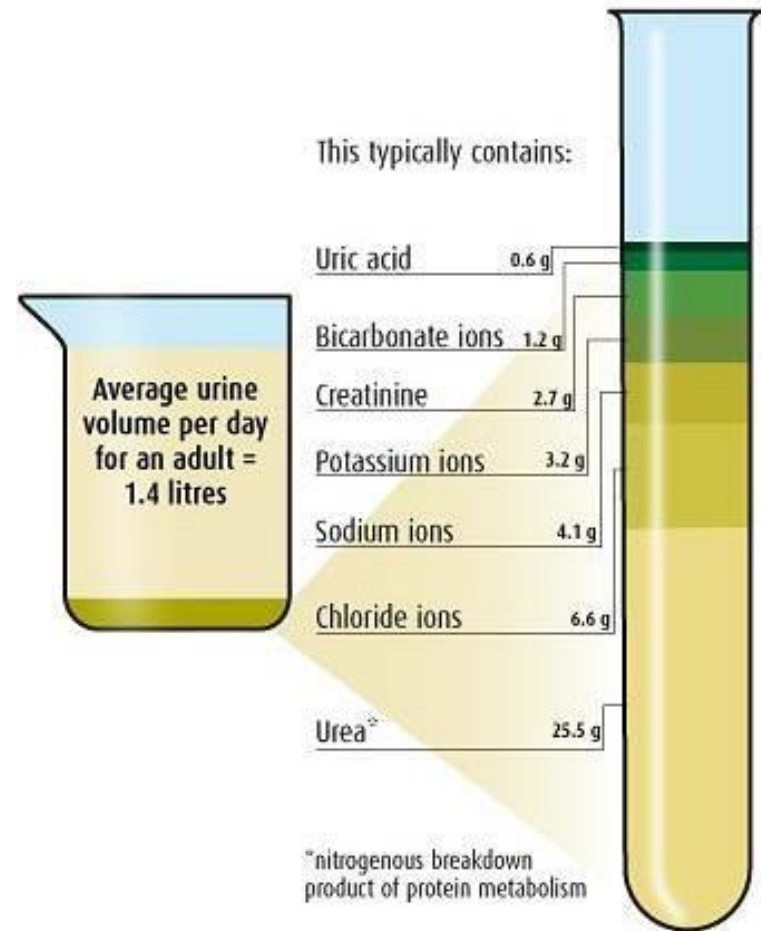
- In the end, **urine** contains substances that have undergone glomerular filtration but have not been reabsorbed and substances that have undergone tubular secretion.
-

Glomerular filtrate vs Urine

Constituent	Daily Excretion	
	Glomerular Filtrate	Urine
Water	130,000 ml	1500 ml
Sodium	20,000 mmol	150 ml
Albumin	4 g (60 μ mol)	0.04 g (6 μ mol)
Urea	900 mmol	400 mmol

Composition of Normal Urine

- Water 96%
 - Urea 2%
 - Uric acid
 - Creatinine
 - Ammonia
 - Sodium
 - Potassium
 - Chloride
 - Phosphate
 - Sulphate
 - oxalate
- 2%



Urinalysis

- Urinalysis (UA) simply means analysis of urine, it is a laboratory test done to detect problems with your body that can appear in your urine.



Urinalysis- Collection of Urine sample

- Should be collected in Clean, dry, wide mouth container.
- Container should be properly labelled.

METHODS-

- Collection of entire voided sample
- Catheterization
- Subrapubic aspiration

PRESERVATIVES-

- Toluene
 - Formalin
 - Thymol
 - Chloroform
-

Urinalysis- Collection of Urine sample

Timing of Collection

- Random sample- sufficient.
 - 1st specimen voided in morning is more concentrated- **preferred**
 - 24 hours urine sample- for quantitative estimation of proteins, sugars, electrolytes, and hormones.
 - 2-3 hours after eating- for Glycosuria
 - Afternoon sample- For urobilinogen
-

Urinalysis

Physical Examination:

Volume, Specific gravity, Color, Appearance, odor, pH.

Chemical Examination :

For Normal Constituents

- **Organic:** Urea, Uric acid, Creatinine.
- **Inorganic:** Chloride, Phosphate, Bicarbonate, Sulphate, Ammonia, Oxalates

For Abnormal Constituents-

- Proteins, Sugar (Glucose & others), Ketone bodies, Bilirubin, Bile salts & Blood
-

Physical Examination: Volume

The daily output of urine on an average diet and normal fluid intake in an adult is between 1000-2000 ml with an average of 1500 ml/day.

- There are several Factors will affected on urinary output :
 - 1)Physiological factors
 - 2)Pathological factors.
- **Physiological factors:** depends on the fluid intake (which is usually a matter of habit) and on the loss of fluid by other routes (primarily sweating which, in absence of fever, depends on physical activity and on the external temperature).



Physical Examination: Volume

Pathological factors:

Polyurea

- More than 2000 ml/day
- Diabetes mellitus
- Chronic renal insufficiency

Oligurea

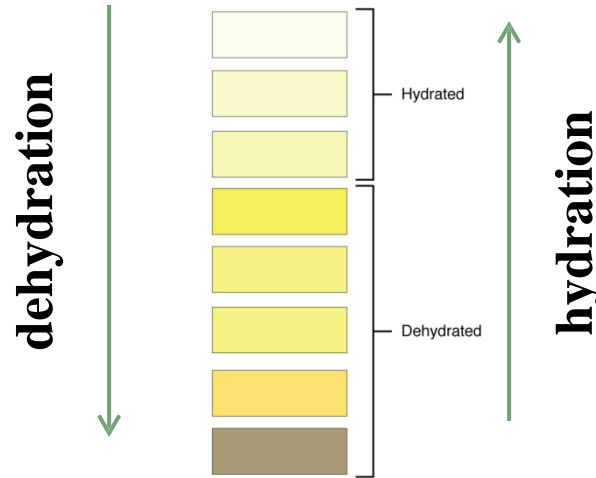
- Below 500 ml /day
- Incase of deficient intake of water or excessive loss of fluids by other routs like hemorrhage or as diarrhea and vomiting

Anurea

- 100 ml /day
 - Stones or tumors in the urinary tract creating an obstruction to urinary flow
-

Physical Examination: Color

- Normally, Urine is clear and amber (yellow) in color due to the presence of **urobilin**



- the **higher** the concentration of urine, the **deeper is the color**.
 - Pale urine has a **low** specific gravity, a dark line has a **high** specific gravity.
 - The concentration of urine is **highest** in the a morning specimen (overnight urine) and is lowest in a specimen passed an hour after much fluid has been taken.
 - Colored urines occur in certain **diseases** or metabolic disorders, and after the administration of many drugs.
-

Physical Examination: Color

Color	Pathological	Non pathological
White	Chyle Pus	Phosphates
Yellow to Orange	Bilirubin Urobilin	Concentrated urine Carrots, Senna Riboflavin Acridine sulfasalazine
Pink to Red	Haemoglobin Myoglobin Porphyrins Red blood cells	Beets (anthocyanin) Aminopyrine Methyldopa Food color Bromosulfonphthalein Pyridium Senna

Physical Examination: Color

	Pathological	Nonpathological
Red to Brown to Purple	Porphobilinogen Uroporphyn	
Brown to Black	Homogenistic acid Melanin Myoglobin Methaemoglobin Phenol Porphyrins	Chloroquine Iron compounds Levodopa Metronidazole Quinine
Blue to Green	Biliverdin Pseudomonas infection	Acriflavine Azure A Methylene blue, Vit B Phenyl salicylate Amitryptiline

Physical Examination: Odor

- Normally Urine smells **aromatic** due to the presence of volatile organic acids.
 - The odor is modified by ingestion of certain foods & drugs. This is noticed after eating asparagus; the odor is due to methyl mercaptan.
 - The urine of patients with **diabetes** mellitus may have a **fruity (acetone)** odor **because of ketosis**.
 - The **ammonical** smell of urine is due to action of bacteria on urea.
 - **Pungent smell-presence** of bacteria/ specimen contaminated with bacteria.
 - **Sweaty feet-** Isovaleric acidemia
 - **Misty/mousy odour-** Phenylketonuria.
 - **Maple syrup-** Congenital metabolic disorder.
 - **Fishy odour/Rancid butter-** Hypermethioninemia
-

Physical Examination: Appearance

- Normal urine is **clear**.
- **Cloudy** Precipitation of amorphous phosphates in alkaline urine / amorphous urates in acid urine. Amorphous phosphates dissolve on addition of acetic acid. Amorphous urates will dissolve when specimen is heated.
- **Turbid**- Leucocytes, epithelial cells, bacteria
- **Hazy**- Mucous
- **Smoky**- RBC
- **Milky**- Fat, Chyle



Physical Examination: pH

On a normal mixed diet the urine is usually **acid**, generally varying in pH between 5.5 and 8.0, with a mean of 6 in 24 hours.

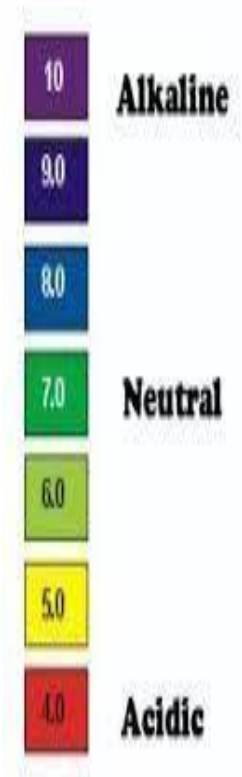
Acidic Urine : Diabetic ketosis, fevers.

Alkaline Urine: A vegetarian diet which causes a tendency to alkalosis. It may also be grossly increased by bacterial infection of the urinary tract.

PROCEDURE

Dip the litmus paper strips in the urine, remove and read the color change immediately.

- Blue litmus turns red – acid
- Red litmus turns blue – alkaline



pH

Decrease in pH

- High protein intake
- Ingestion of cranberries
- Respiratory acidosis
- Metabolic acidosis
- Uremia
- Severe diarrhoea
- Starvation
- UTI caused by E. coli

Increase in pH

- Diet high in vegetables and citrus fruits
 - Respiratory alkalosis
 - Metabolic alkalosis
 - Vomiting
 - UTI caused by Proteus and Pseudomonas
-

Physical Examination: Specific Gravity

- SG is a measure of the density of the dissolved chemicals in the specimen.
 - It is Ratio of weight of a volume of urine to the weight of the same volume of distilled water at a constant temperature.
 - Measure the concentrating and diluting power of kidney.
 - There are **direct relation ship** between concentration of substance in urine (Concentration of urine) and SG.
 - Specific gravity increases when fluid intake is low and decreases when fluid intake is high.
 - The normal specific gravity (correctly called relative density) of a pooled 24 hour urine sample is between 1.003 and 1.030 .
-

Physical Examination: Specific Gravity

- **Hyposthenuria**
 - Consistently low specific gravity, <1.007 .
 - Due to concentration problem.
 - **Hypersthenuria**
 - Consistently high specific gravity
 - Due to deprivation of water.
 - **Isosthenuria**
 - Fixed specific gravity of 1.010
 - Indicates poor tubular reabsorption
-

Urinometer

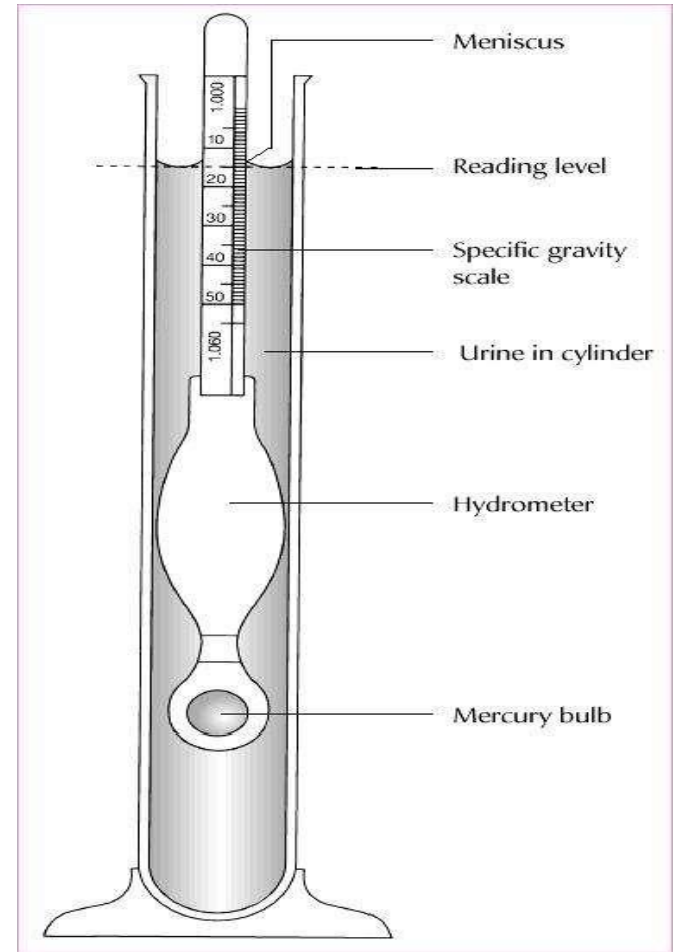
- It is a hydrometer that is calibrated to measure the specific gravity of urine at a specific temperature, usually at 20°C.
- Based on principle of buoyancy so the urinometer will float higher in urine than in water, because urine is denser.
- Thus higher the specific gravity of a specimen, the higher the urinometer will float.
- Specific gravity is affected by presence of dense molecules, protein and glucose.
- Subtract **0.03** from specific gravity after temperature correction for each 1 g/dl of **protein** and **0.004** for each 1g/dl of **glucose**.

Temperature correction-

- For every 3°C below 20°C, subtract 0.001 from the reading and for every 3°C above 20°C, add 0.001.
-

Urinometer

1. Allow urine to reach room temperature.
2. Check urinometer periodically with distilled water to see if its read 1.000.
3. Mix urine
4. Add to cylinder (approx 15 ml).
5. Remove any foam because bubbles interfere with the reading of meniscus.
6. The hydrometer must not come in contact with the bottom or the sides of the cylinder.
7. Allow it to float freely.
8. It is necessary to spin the urinometer so that it will float in the center of the cylinder.
9. Read the bottom of the meniscus while looking at the hydrometer at eye level.



Urinometer

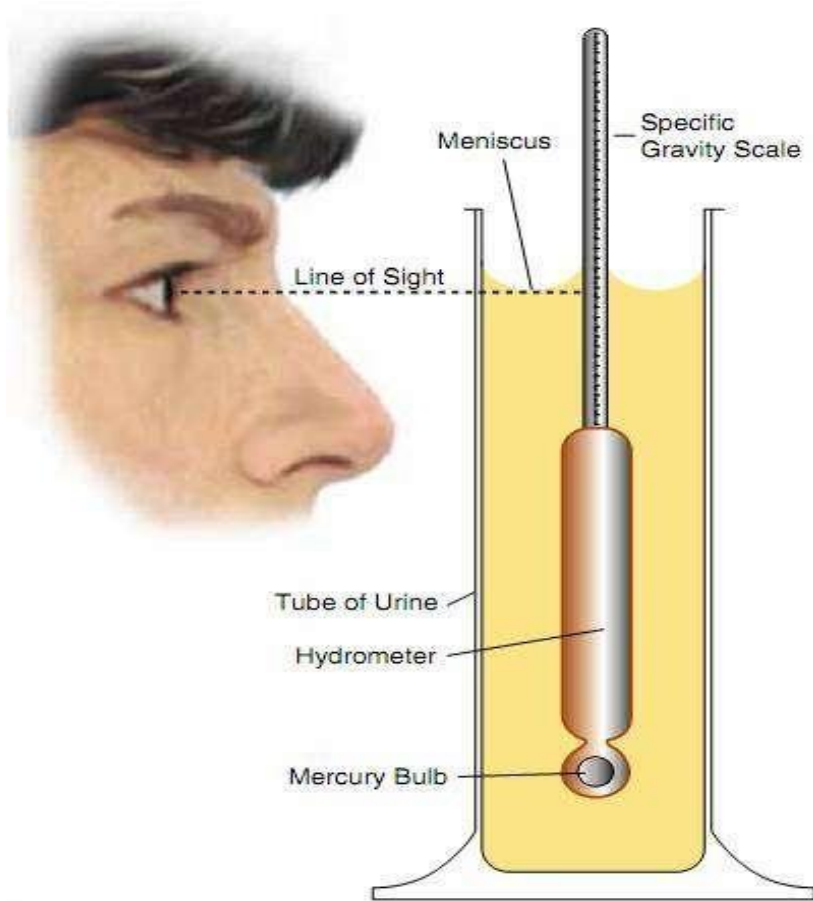


Figure 28-12 Urinometer



Urinometer



Chemical Examination:

- A series of chemical tests is run.
- Usually, A chemically impregnated **dipstick** can be used for many of these tests .
- These urinalysis **test strips** (dip sticks) have small test patches impregnated with **various chemicals** in order to detect the presence or absence of certain substances. Qualitative and/or quantitative results can be obtained depending on the particular test.



Test strips (dipsticks)

- The test strips consist of absorbent microfiber cellulose pads attached to it.
- Each pad contains the dried reagents needed for a specific test that react with the compounds present in urine producing a characteristic **color**.
- There are strips which serve different purposes, such as **qualitative** strips that only determine if the sample is positive or negative, or there are **semi-quantitative**. **Semi-quantitative strips** provide an estimation of a quantitative result, the color reactions are approximately proportional to the concentration of the substance being tested for in the sample.
- The reading of the results is carried out by comparing the pad colors with a color scale provided by the manufacturer.



How to test your urine(visual read)?



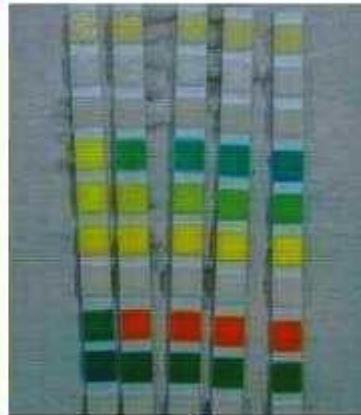
A

Prepare some fresh urine sample.



B

Dip the dry strip into the urine.



C

Absorb the excess urine with absorbent paper.



D

Contrast color chart, close to which color?

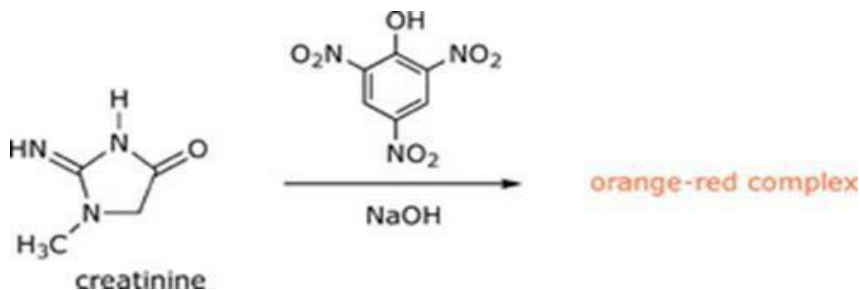
Chemical Examination for Normal constituents

Organic Constituents

- **Uric acid:**
- To 2 ml of urine add 1 ml of Benedict's reagent, then heated in a boiling water bath for three minutes. **white precipitate** indicates the presence of uric acid.



- **Creatinine:**
- To about 5 ml of urine add a few drops of a saturated solution of picric acid. On rendering the solution alkaline with a few drops of 10% sodium hydroxide solution, a deep **red color or orange** due to creatinine picrate appears.



Inorganic constituents

Chloride:

- 5 ml of Urine +5 drops of 2N nitric acid+2N silver nitrate solution.
- A **white precipitate** of silver chloride is formed.
- Silver chloride is precipitated in the presence of nitric acid and silver nitrate.



Phosphate:

- 5 ml of urine +5ml nitric acid+4 ml of sodium molybdate -----heat.
- A **yellow crystalline** precipitate of ammonium phospho-molybdate appears.



Bicarbonate:

- 4 drops of concentrate hydrochloric +5 ml of urine.
- A slight **effervescence** occurs due to CO₂ evolution.

Sulphate:

- Acidify 10 ml of urine with 1ml dilute hydrochloric acid + 4 drops of 5% barium chloride solution.
- A **white precipitate** sulphate is precepitated as of barium sulphate is formed.



Ammonia:

- 1 ml of 10% sodium hydroxide solution +5 ml or urine. Boil.
- The evolved ammonia may be detected by turning moist **red litmus paper blue.**



Test For	reagent	Color
Creatinine	saturated solution of picric acid in alkaline condition	Red-orange color
Uric acid	Benedict reagent after heating	White precipitate
Chloride	nitric acid and silver nitrate	White precipitate
Phosphate	concentrated nitric acid and saturated ammonium molybdate	Yellow precipitate
Bicarbonate	concentrate hydrochloric acid	gaseous carbon dioxide.
Sulphate	dilute hydrochloric acid + 1 ml drops of 5% barium chloride solution	white precipitate
Ammonia	sodium hydroxide	ammonia gas with sodium hydroxide . This is an alkaline gas . It turns red litmus paper blue

Chemical Examination for Abnormal Constituents

Abnormal Constituents-

- 1-Proteins
 - 2-Sugar(Glucose & others)
 - 3-Ketone bodies
 - 4-Bile salts
 - 5-Bile pigments
 - 6-Blood
-

Proteins

- Normal- upto 150 mg/24 hours or 10mg/100ml in single sample.

Tests (Qualitative)

- **Heat Coagulation and acetic acid test-** The test is based on the principle of heat coagulation and precipitation of proteins by acetic acid.
 - **Sulphosalicylic acid test-** Sulphosalicylic acid neutralizes protein cation, resulting in precipitation.
 - **Heller's Nitric Acid Ring test** is a chemical test that shows that strong acids cause the denaturation of precipitated proteins. Concentrated nitric acid is added to a protein solution from the side of the test tube to form two layers. A white ring appears between the two layers if the test is positive. Heller's test is commonly used to test for the presence of proteins in urine.
-

Proteins

Procedure of Qualitative tests-

1- Heat Coagulation & Acetic acid test-

- Take a long test tube and fill $\frac{3}{4}$ the tube with clear urine.
- Boil the upper portion over a flame, the lower portion serves as the control.
- If proteins, phosphates or carbonates are present in the urine a turbidity develops.
- Add 1-3 drops of 10% glacial acetic acid.
- Any turbidity due to phosphate precipitation will clear or if it is due to carbonates they disappear with effervescence.
- If it persists, it is due to albumin.

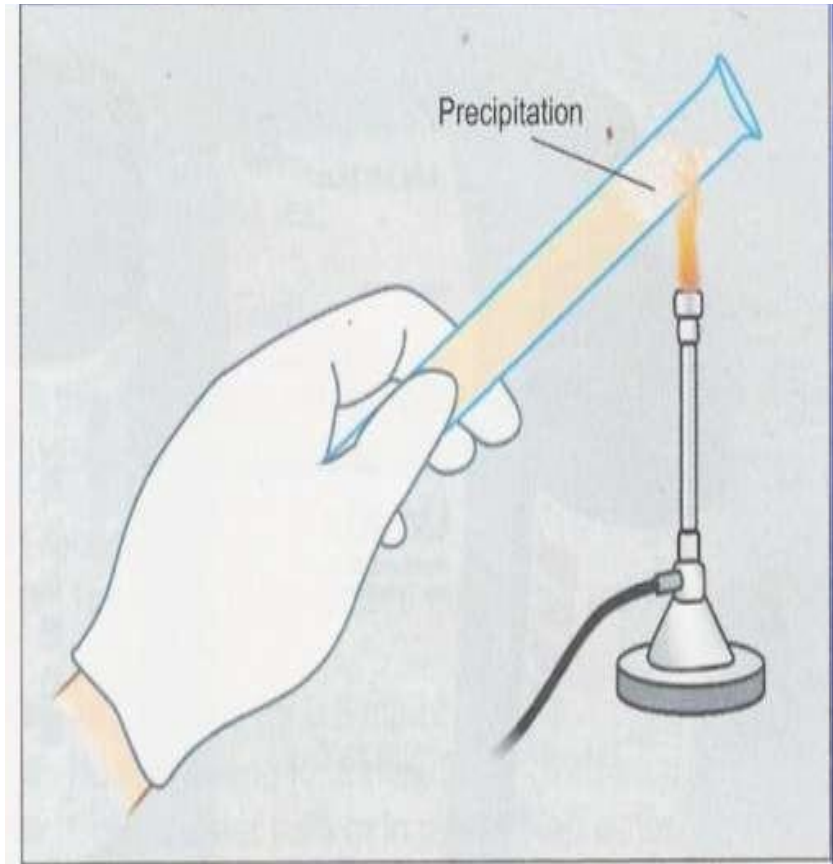
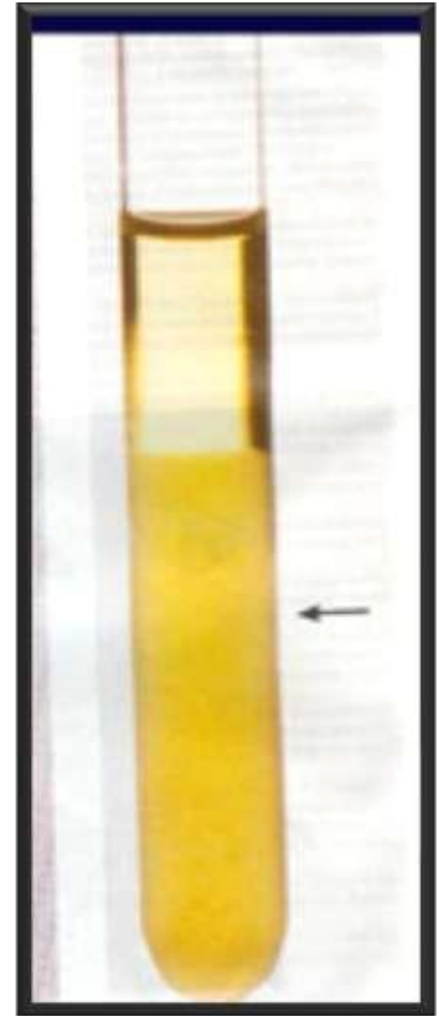


FIGURE 4.2: Heat and acetic acid test for proteinuria. Note the method of holding the tube from the bottom while heating the upper part.

Proteins

Heat Coagulation test- Interpretation

- ❑ Negative – No turbidity or cloudiness.
- ❑ Trace – Cloudiness visible against a black background (5 mg / dl).
- ❑ 1+ -Definite cloudiness without flocculation and granularity (10 – 30 mg / dl).
- ❑ 2+ -Heavy and granular cloudiness without flocculation (40 – 100 mg / dl).
- ❑ 3+ -Dense opaque cloud with marked flocculation (200 – 500 mg / dl) .
- ❑ 4+ -Thick cloudiness with precipitation



Proteins

Sulphosalicylic acid test-

- Take 2ml of acidic urine taken in a test tube.
 - Add an equal volume of 20% Sulphosalicylic acid.
 - Mix thoroughly, allow it to stand for 10 minutes and estimate the amount of turbidity.
 - Absence of cloudiness- Absence of protein.
 - **If turbidity persists after boiling- Positive for protein.**

 - Negative : No cloudiness
 - Trace: Barely visible cloudiness.
 - 1+ : definite cloud without granular flocculation
 - 2+ : heavy and granular cloud without granular flocculation
 - 3+ : dense cloud with marked flocculation.
 - 4+ : Cloudiness with precipitation
-

Proteins

3-Heller's Nitric Acid Ring test

- Take urine sample in the test tube.
 - Add Concentrated Nitric acid solution from the side of test tube to form two layers.
 - A white ring appears between the two layers if the test is positive.
-

Proteinuria

Pre-renal

- Addison's disease
- Fever
- Eclampsia
- Hypertension
- Haemoglobinuria
- Rhabdomyolysis

Renal

- All cases of glomerulonephritis
- Nephrotic syndrome
- Pyelonephritis

Post renal

- Lesions of renal pelvis, urethra (cystitis, prostatitis)
 - Severe UTI
-

Proteinuria

Minimal Proteinuria

(<0.5 gm/day)

- Exercise
- Fever
- Emotional stress
- HTN
- Renal tubular dysfunction
- Polycystic kidneys
- Lower UTI

Moderate proteinuria (0.5-3 gm/day)

- Chronic glomerulonephritis
- CCF
- Pyelonephritis
- Pre-eclampsia
- Multiple myeloma

Marked Proteinuria (> 3gm/day)

- Acute glomerulonephritis
 - Chronic glomerulonephritis, severe
 - Nephrotic syndrome
 - Diabetic nephropathy, severe
 - Renal amyloidosis
 - Lupus nephritis
-

Quantitative estimation of Protein in urine

Esbach's method using albuminometer

Reagents-

- Esbach's reagent-Picric acid, Citric acid, Water
- Acetic acid
- pH paper

Instrument

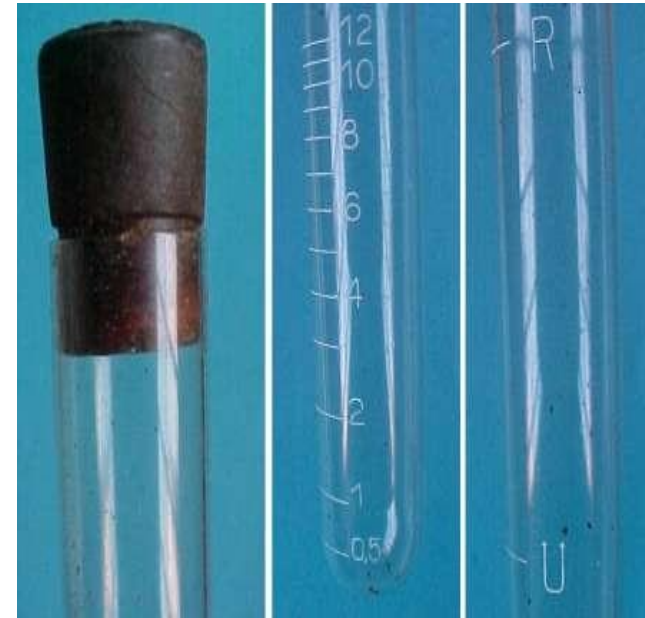
- Esbach's albuminometer



Protein

Procedure- Esbach's Method

- Fill Esbach's albuminometer with acidic urine upto mark U and reagent is added upto mark R.
- Tube is shaken well by inversion.
- Stopper the tube.
- Keep in standing erect position for 18-24 hours for the precipitate to settle down.
- Reading of the length of ppt is taken indicated by markings present over the tube.
- Albumin is expressed in gm/L of urine.
- When test done on 24 hours urine sample, quantity of urine passed per day may be calculated by Dividing quantity of albumin per litre by total quantity of urine passed in 24 hours in litre.



Microalbuminuria

- Urinary albumin excretion between 30-300 mg/day.
 - Cannot be detected by dipstick methods.
 - Strong predictor of development of diabetic nephropathy.
 - Can be detected 10-15 years before development of diabetic nephropathy.
 - Significant risk marker of cardiovascular disease.
 - **Diagnostic relevance**
 - In diabetic patients for early diagnosis of nephropathy.
 - In hypertensive patients as indicator of end organ damage
-

Bence-Jones proteins

- BJ protein is abnormal LMW globulin consisting of light chains of Ig either Lambda or Kappa chains.
- Characteristic feature- Precipitated at 40⁰ C to 60⁰ C temperature and redissolves at higher temperature (100⁰c) & reappears when the urine is cooled

Conditions

- Multiple myeloma
 - Plasmacytoma
 - Waldenström macroglobinaemia
-

Detection of Bence Jones Proteins

- Take 5ml urine in a test tube.
 - If the urine is cloudy, than filter it with filter paper.
 - If the reaction is alkaline of urine than do it acidic by adding a few drops of 25% acetic acid.
 - Than set the test tube in a water bath.
 - Heat in water bath for 15 minutes.
 - If the Bence -Jones Protein is present in urine then precipitate forms between temperature of 40°C -60°C.
 - But when temperature is raised to 85^o -100°C, precipitate disappears.
 - When the temperature is decreased to 60°C, precipitate reappears.
 - It again disappears when temperature goes below 40°C.
-

Sugars

- This is a non-specific test useful for semiquantitation of marked glucosuria.

Benedict's qualitative test

- Principle- Aldehyde group of reducing sugar reduces Cupric ions in Benedict's reagent to cuprous oxide.
- Detects all sugars except sucrose.
- The final color of the solution depends on how much of this precipitate was formed, and therefore the color gives an indication of how much reducing sugar was present.

Increasing amounts of reducing sugar

- **Green yellow orange red**
-

Sugars

Benedict's test

Components :

- **Sodium carbonate-** 100 gm (Provides alkaline conditions which are required for the redox reaction)
- **Sodium citrate-** 173 gm (complexes with the copper (II) ions so that they do not deteriorate to copper(I) ions during storage)
- **Copper sulphate-** 17.3 gm

Procedure

- Take 5ml of Benedict's reagent
 - Boil for 3 – 5 minutes
 - Add 0.5ml (8 drops) of urine.
 - Boil for 2 minutes.
 - Cool and note the colour.
-

Sugars

Benedict's test

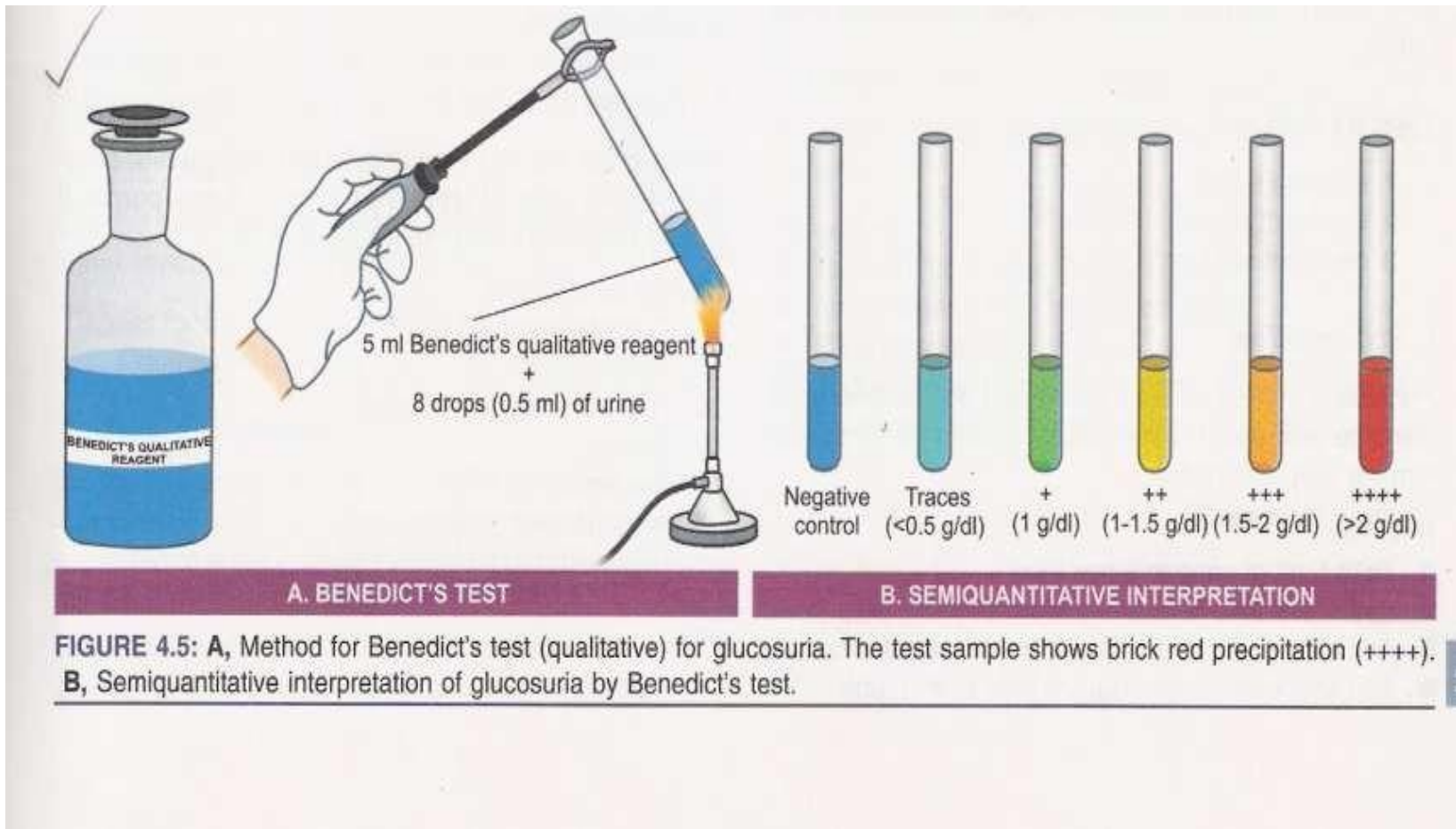
Recording results

The color varies from blue through green – yellow- orange- brick red.

- | | |
|------------|----------------------------------|
| ➤ Negative | No change in color. |
| ➤ Trace | Greenish blue |
| ➤ 1+ | Greenish yellow (0.5% sugar) |
| ➤ 2+ | Yellow (1% sugar) |
| ➤ 3+ | Orange precipitate (1.5% sugar) |
| ➤ 4+ | Brick red precipitate (2% sugar) |
-

Sugars

Benedict's Test



Sugars detected by Benedict's Test

- Glucose
- Galactose
- Lactose
- Fructose
- Maltose
- Pentose

False +ve Benedict's test by

- Ascorbic acid
 - Creatinine
 - Uric acid
 - Salicylates
-

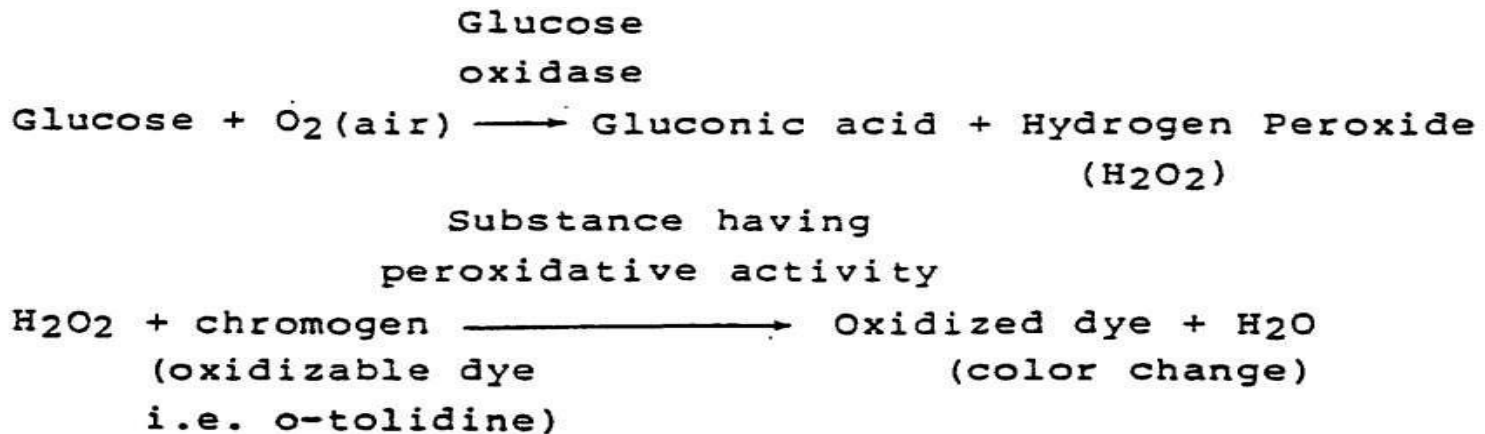
Benedict's test

Principle:

This test is based on a double sequential enzyme reaction.

One enzyme, glucose oxidase, catalyzes the formation of gluconic acid and hydrogen peroxide from the oxidation of glucose.

A second enzyme, peroxidase catalyzes the reaction of hydrogen peroxide with potassium iodide chromogen to oxidize the chromogen to colors ranging from green to brown.



Glycosuria

Definition

The condition in which abnormal quantities of glucose are excreted in urine is called Glycosuria.

Normal urine contains traces of glucose which can not be detected by benedict's test. Beyond the renal threshold value- 160-180 mg/100ml, the tubules can not reabsorb glucose which escapes reabsorption and is excreted in urine.

Occurs in two conditions

In normal blood glucose level

In hyperglycemia

Glycosuria

A-In normal blood glucose level

- | | |
|-----------------------------|---------------------------------|
| 1- Alimentary Glycosuria | 2- Emotional Glycosuria |
| 3- In pregnancy & Lactation | 4-Renal (hereditary) Glycosuria |

1-Alimentary Glycosuria

Some person excrete glucose in urine after the intake of large amounts of sugar or carbohydrate rich meal in spite of their normal blood glucose level. They have normal renal threshold for glucose but their blood glucose level shoots up(200-220mg/100ml) for a short period. Hence a **transitory glycosuria** occurs.

2-Emotional Glycosuria-

Occurs in periods of excessive nervous strain & emotional excitement such as intense fear, anger and severe anxiety due to increased secretion of epinephrine. It has been observed in college students appearing in the examination, worried athletes and candidates for competitive examinations.

Glycosuria

3- In pregnancy & lactation

- Occurs in normal pregnant women in later months due to temporary reduction in maximum tubular reabsorption capacity of glucose and partly due to decreased glucose tolerance caused by temporary hypertrophy of the pituitary gland.

4- Renal (Hereditary) Glycosuria

- Some persons have low renal threshold value for glucose which may be below 150mg/100ml.
 - This condition is harmless.
 - Also known as Diabetes innocens or benign hypoglycosuria.
 - Such persons have an impaired tubular reabsorption for glucose.
 - It is hereditary defect.
-

Glycosuria

Standards for the diagnosis of true Renal Glycosuria

- 1- Normal blood glucose level in fasting
- 2- glucose is present in every sample of urine, either in fasting state or after a meal.
- 3- Normal carbohydrate utilization
- 4- No disturbance of fat metabolism.
- 5- Moderate doses of insulin have little or no effect upon the glycosuria.

Note-

Practical danger in making the diagnosis of renal glycosuria lies in the confusion of this condition with diabetes mellitus.

No metabolic disturbance occurs in subjects with renal glycosuria as long as the carbohydrate intake is adequate to compensate for the amount lost in the urine.

Glycosuria

B-Glycosuria in hyperglycemia

- Occurs due to increased blood glucose level in Diabetes Mellitus.
 - Blood glucose level becomes very high and Glucose is excreted in the urine.
 - In this renal threshold value for glucose is normal.
-

Ketone bodies

Ketone bodies-

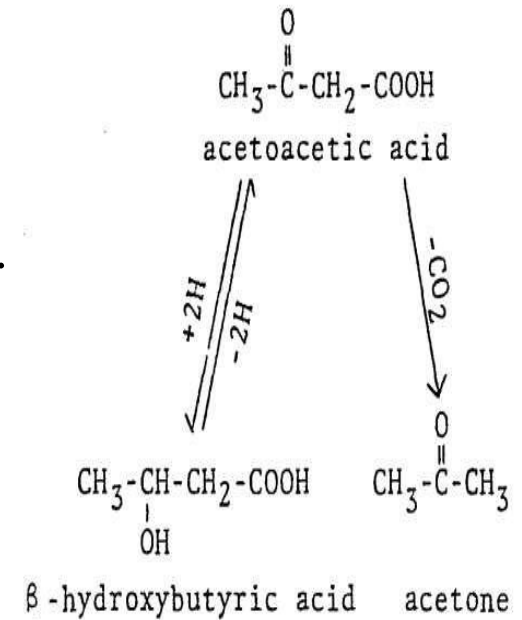
- 1- Acetoacetic Acid
- 2- Acetone
- 3- Betahydroxybutyric acid (False ketone body).
It does not give Rothera's Test.

Ketonuria-

It is condition when ketone bodies are excreted in urine.

Causes-

- Diabetes Keto Acidosis
- Fever
- Anorexia
- Gastrointestinal disturbances
- Fasting
- Starvation
- Severe vomiting

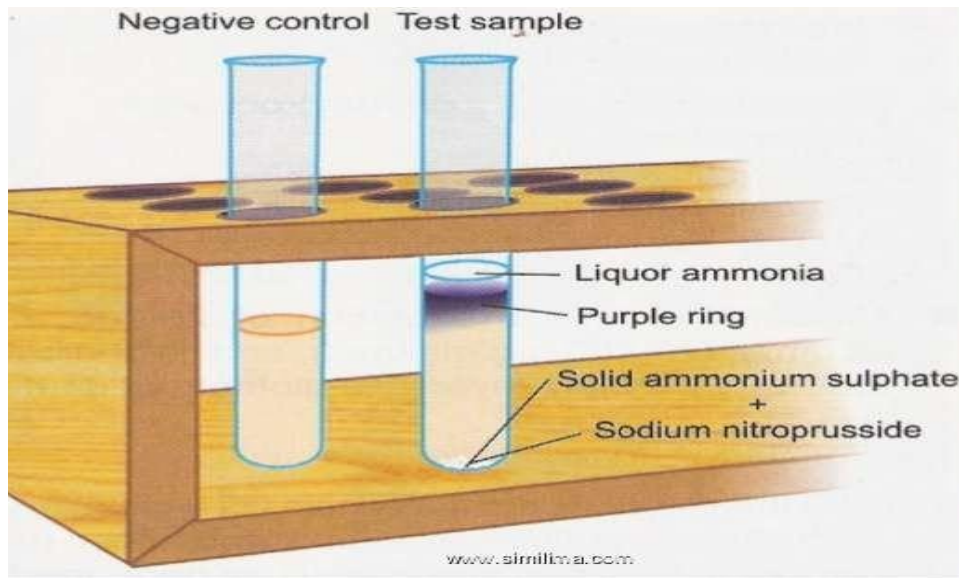


Test for ketone bodies-Rothera's test

Procedure:

- Take 5ml of urine in a test tube and saturate it with ammonium sulphate.
- Add 1 crystal of sodium nitroprusside.
- Mix.
- Run liquid ammonia carefully at the side of the tube so as to form a layer on top of the saturated urine.

Positive- Formation of purple ring at junction of two fluids.



Bile salts

Primary bile acids

- Cholic acid and chenodeoxycholic acid (CDCA)- synthesized from cholesterol in the liver, conjugated with glycine or taurine, and secreted into the bile.

Secondary bile acids

- Deoxycholate and lithocholate, are formed in the colon as bacterial metabolites of the primary bile acids.
 - **Bile salts- Sodium taurocholate and sodium glycocholate are found in urine.**
-

Tests for Bile salts in urine

Hay's Sulphur Test

Principle:

- Bile salts when present decreases surface tension of urine.

Procedure:

- Take 10 ml of urine in beaker.
- Sprinkle dry sulphur powder on the surface of the urine

Result:

- If bile salts are present they sink to the bottom.
 - Otherwise they float on the surface.
-

Bile pigments

In Normal urine-

- Urochrome
- Traces of Urobilin

In Abnormal Urine-

- Bilirubin
 - Urobilinogen
 - Biliverdin
 - Urobilin
-

Bile pigments

Fouchet's Test:

Fouchet's Reagent

- Trichloroacetic acid – 25 gms
- Distilled water - 100 ml
- 10% Ferric chloride solution – 10 ml.

Principle:

- Barium chloride added to urine combines with sulphate radicals in urine to form precipitate of barium phosphate. If bile pigments are present in urine, they will adhere to these large molecules.
 - Ferric chloride present in fouchet reagent then oxidizes yellow bilirubin in presence of trichloroacetic acid to green biliverdin.
-

Bile pigments

Ehrlich's test for urobilinogen

Principle-

- Urobilinogen reacts with p-dimethylamino- benzaldehyde to form red colour.
Intensity of red colour is proportional to the concentration of urobilinogen in urine.

Reagents-

- P-dimethylaminobenzaldehyde, HCL, Distill Water

Procedure-

- Add 1ml of Ehrlich's reagent to 10 ml of urine in test tube.
- Mix by inversion. Let stand for 5 minutes.

Result

- Pink- Normal
- Dark red colour- Positive for urobilinogen.

Causes of increased urobilinogen-

- Haemolytic jaundice, Pernicious Anemia, Paralytic enterocolitis & Hepatic congestion
-

Blood

- In the lesion of kidney or urinary tract blood is excreted in the urine.
 - Free haemoglobin is also found in urine after quick hemolysis e.g. in black water fever(a complication of malaria) or after severe burns.
-

Blood

Haematuria- when 5 or more intact RBCs/HPF.

Causes-

Renal

- Neoplasms
- Calculi
- TB
- Pyelonephritis
- Hydronephrosis
- Oxaluria
- Acute GN
- Polycystic kidney disease

Post Renal

- Ureter- calculus, neoplasm
- Urinary bladder- neoplasm, TB, Cystitis, calculus.
- Prostate- BPH, Neoplasm

General

- Embolism of kidney from SBE.
 - Malignant HTN kidney
 - Haemophilia
-

Blood

Test

Benzidine Test

Procedure

- Add 2 ml of urine in test tube.
- Add 2ml of 1% Benzidine solution in acetic acid.
- Shake well.
- Add 2ml of hydrogen peroxide.
- Mix and observe for a change in color.

Positive result: Green or blue color. Hematuria +nt

Test Strips (Dipsticks)



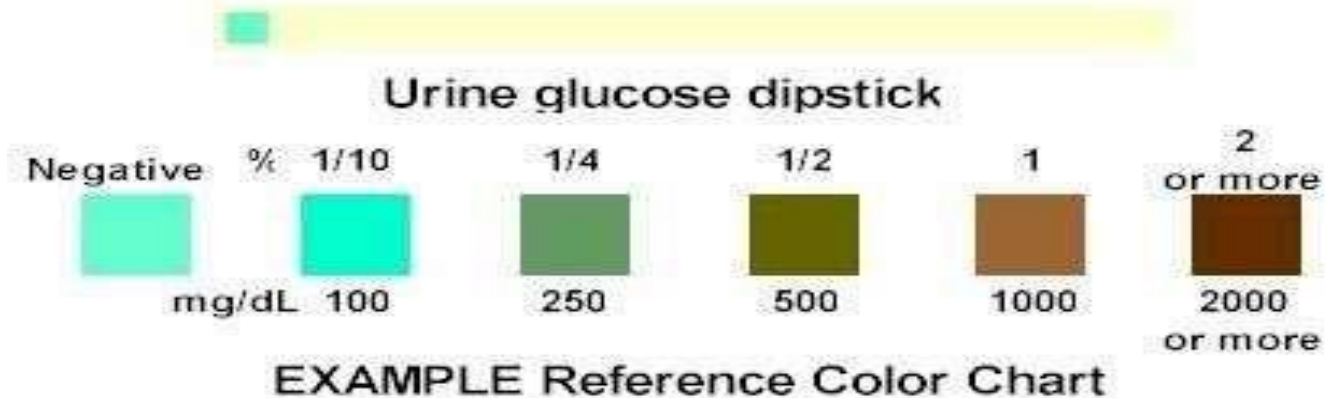


Test strips (Dipsticks)

TESTS AND READING TIME

LEUKOCYTES 2 minutes	NEGATIVE	TRACE	SMALL +	MODERATE ++	LARGE +++		
NITRITE 60 seconds	NEGATIVE	POSITIVE	POSITIVE	(key to level of oxidant present in specimen)			
UROBILINOGEN 60 seconds	NORMAL 1.5	NORMAL 1	SMALL 2	4	8 17 mg = 350000 IU		
PROTEIN 60 seconds	NEGATIVE	TRACE	100 ++	300 +++	2000 or more ++++		
pH 60 seconds	5.0	6.0	6.5	7.0	7.5	8.0	8.5
BLOOD 60 seconds	NEGATIVE	TRACE	SMALL 1	MODERATE 2	SMALL 4	MODERATE 6	LARGE 8
SPECIFIC GRAVITY 45 seconds	1.000	1.005	1.010	1.015	1.020	1.025	1.030
KETONE 40 seconds	NEGATIVE	SMALL 5	MODERATE 10	MODERATE 40	LARGE 80	LARGE 160	
BILIRUBIN 30 seconds	NEGATIVE	SMALL +	MODERATE ++	LARGE +++			
GLUCOSE 30 seconds	NEGATIVE	SMALL 100	100 250	500	1000	2000 or more	

Example Diastix urine glucose test strip and color chart





**THANK
YOU**