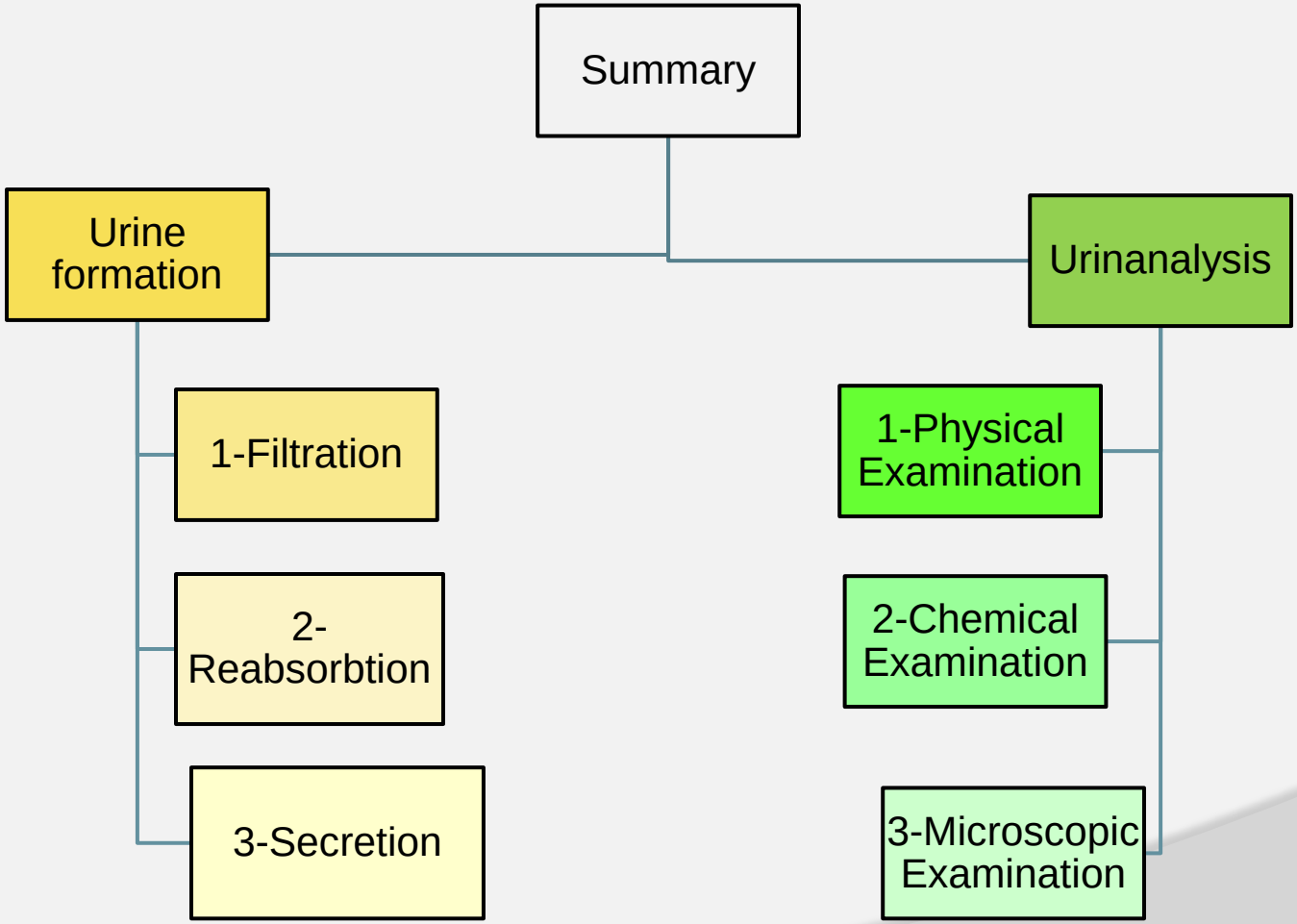
PHYSICAL PROPERTIES AND DETECTION OF NORMAL CONSTITUENTS OF URINE

Objectives

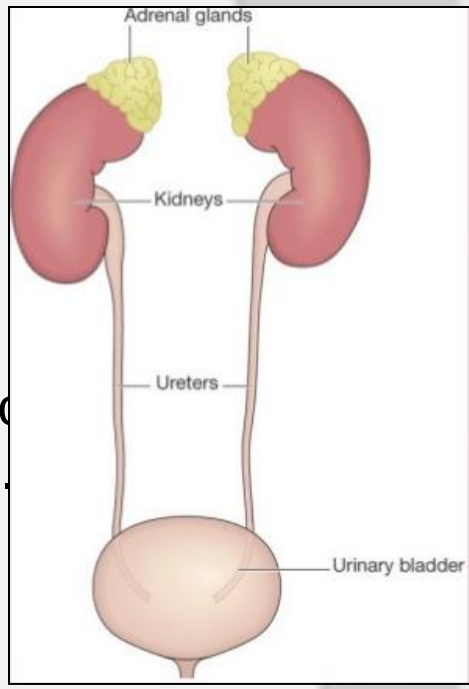
- Simple examination of normal urine
- To detect some of the normal organic constituents of urine
- To detect some of the normal inorganic ions present in urine

Lab Summary


Summary
Urine formation
Urinanalysis
1-Filtration
2-Reabsorbtion
3-Secretion
1-Physical Examination
2-Chemical Examination
3-Microscopic Examination

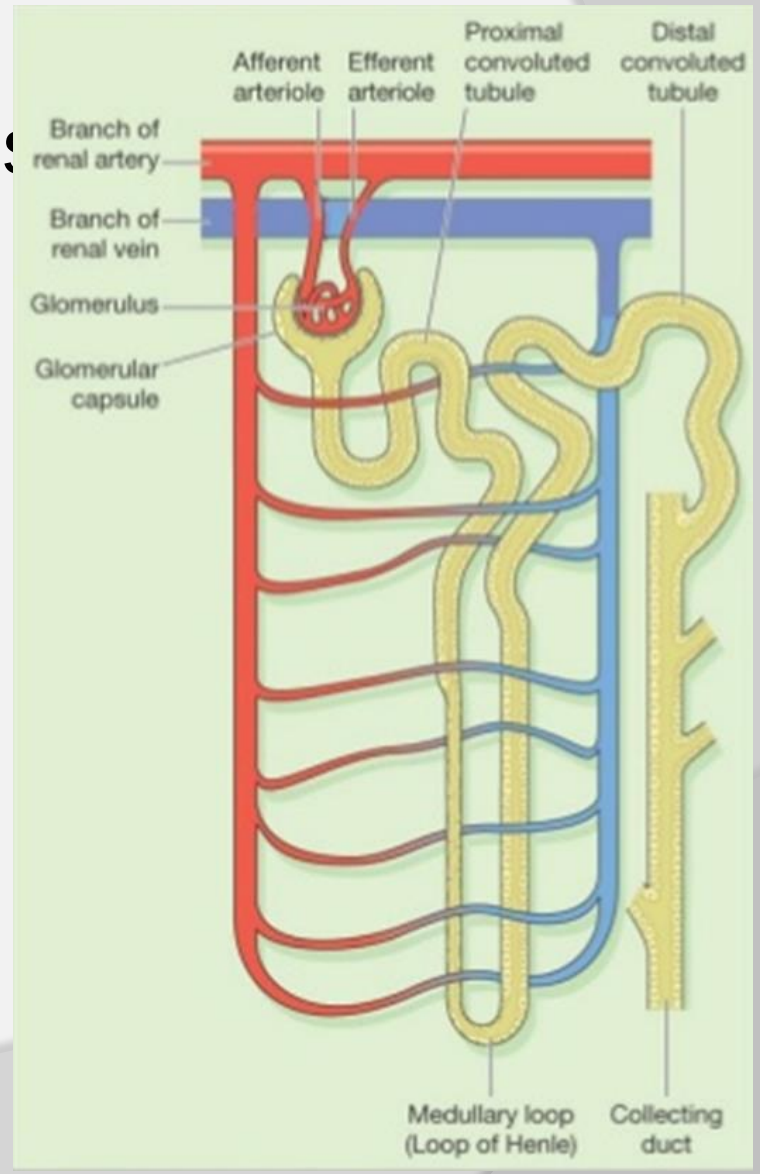
Kidney and Urine

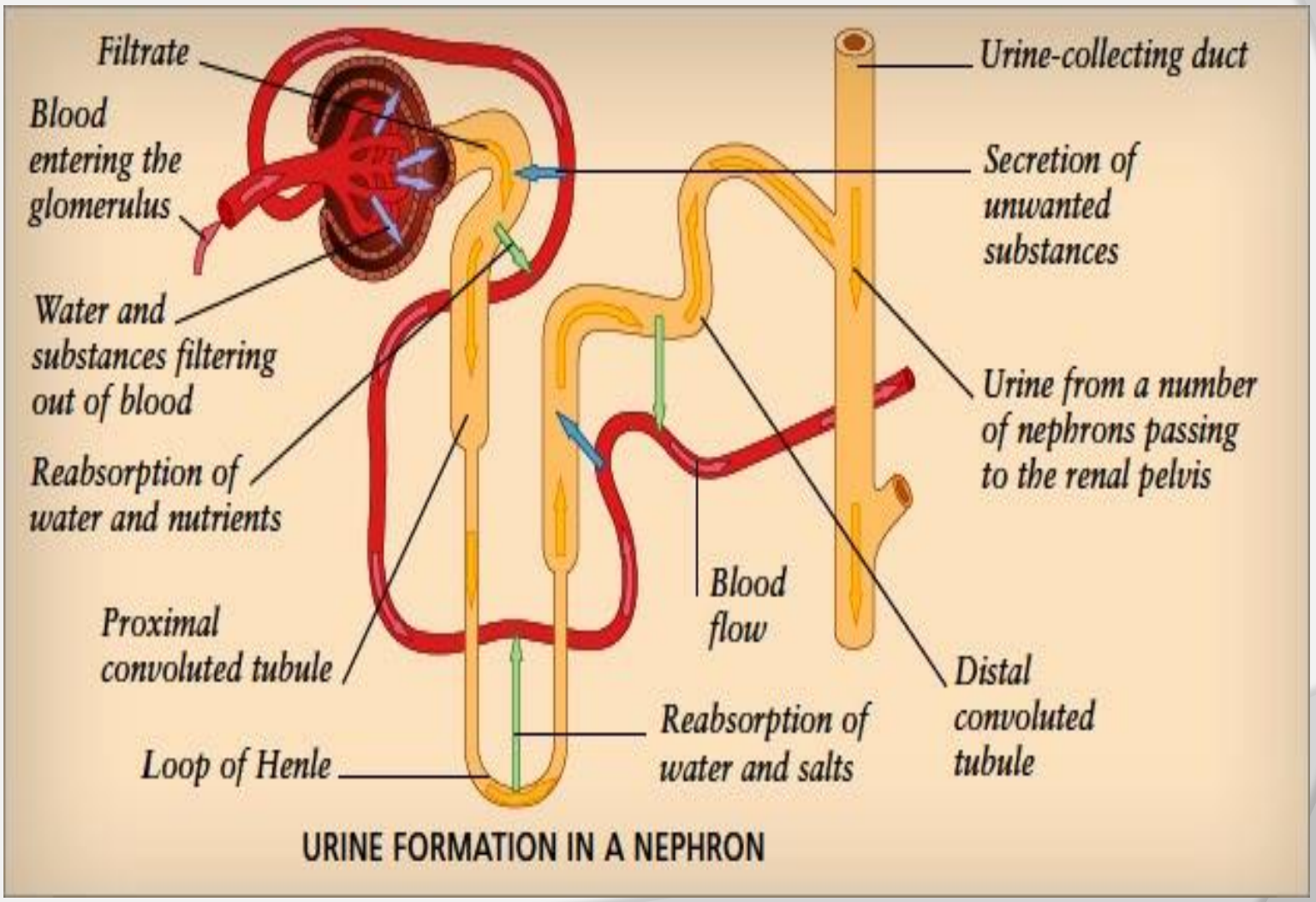
- The kidneys form urine, which passes through the ureters to the bladder for storage prior to excretion.
- The composition of urine reflects exchange of substanc between the nephron and blood in the renal capillaries.
- Waste product of protein metabolism are excreted, electrolyte levels are controlled and pH (acid-base balance) is maintained by excretion of hydrogen ions



Urine Formation

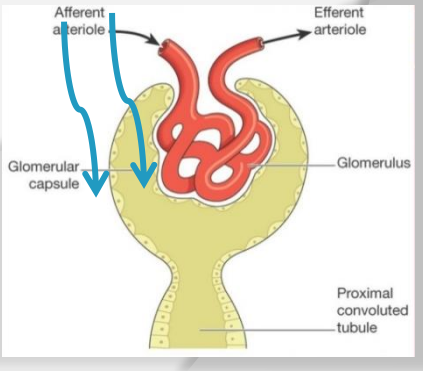
- There are three processes the formation of urine:
- Filtration
- Selective reabsorption
- Secretion




Filtrate
Blood entering the glomerulus
Water and substances filtering out of blood
Reabsorption of water and nutrients
Proximal convoluted tubule
Loop of Henle
Urine-collecting duct
Secretion of unwanted substances
Urine from a number of nephrons passing to the renal pelvis
Blood flow
Distal convoluted tubule
Reabsorption of water and salts
URINE FORMATION IN A NEPHRON

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-Reabsorption

- Is the process of reclaiming water and some solutes from the tubular fluid and returning them to the blood
- 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-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
- Hydrogen ions (H^+), creatinine, and drugs such as penicillin are some of the substances moved by active transport from blood into the kidney tubule

- In the end, urine contains substances that have undergone glomerular filtration but have not been reabsorbed and substances that have undergone tubular secretion. Tubular secretion is now known to occur along the length of the kidney tubule.

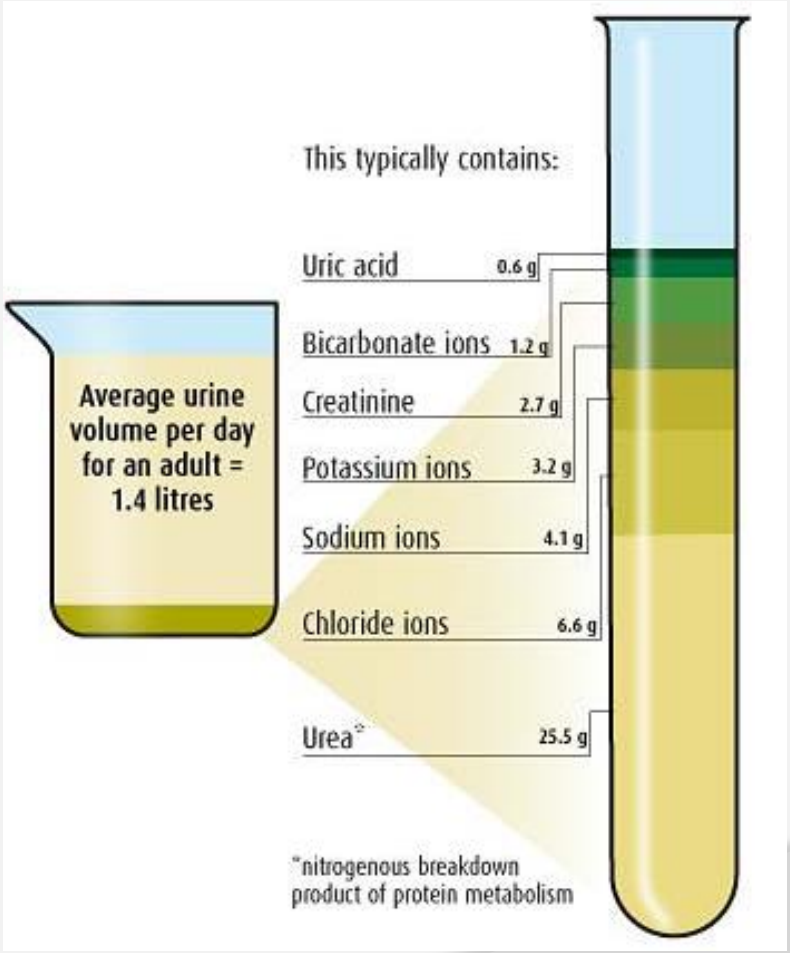
Glomerular filtrate vs Urine

Constituent	**Daily Excretion**	
	Glomerular Filtrate	**Urine**
Water	130,000 ml	1500 ml
Sodium	20,000 mmol	150 ml
Albumin	4 g (60 µmol)	0.04 g (6 µmol)
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 (UA)

- 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.
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Urinalysis

- a complete urinalysis involves an examination of the physical characteristics of urine, a chemical analysis and a microscopic examination of urine sediment.

Collection of urine sample

Type of Specimen	Charectarestic
Random urine specimen (collected by any time)	Good for chemical screening, routine screening, microscopic screening
Fasting or first morning specimen	Best for nitrate, protein pregnancy test, microscopic screening
Clean catch specimen	Bacterial culture
Time specimen –eg 24 hours	Necessary for accurate quantitative result, Chemical testing

Simple Examination of the Urine

- **Physical Examination :**
- Volume, Specific gravity, Color, Appearance, odor, pH

- **Chemical Examination :**
- **Organic:** Uric acid, Creatinine
- **Inorganic:** Chloride, Phosphate, Bicarbonate, Sulphate, Ammonia

PHYSICAL CHARACTERISTICS OF URINE

- The physical characteristics of urine include observations and measurements of Volume, turbidity, color,odor, pH and specific gravity.
- Physical observation of a urine sample can give important clues as to evidence of pathology.

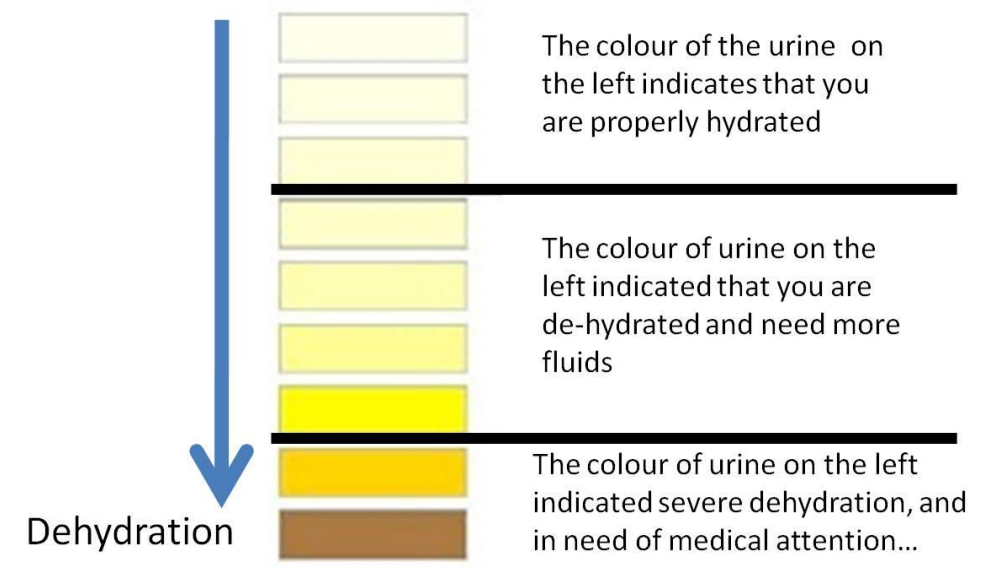
Volume

- The daily output of urine on an avaege diet and normal fluid intake is between 800-2500 ml with an average of 1500 ml/day

polyurea	oligurea	Anurea
• More than 2500 ml/day • Diabetes mellitus • Chronic renal insufficiency	• Below 500 ml /day • Incase of deficient intake of water or excessive loss of fluids by other routs like haemorrhage or as diarrhea and vomting	• 100 ml /day • Stones or tumours in the urinary tract can also cause it by creating an obstruction to urinary flow

Color

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



- Pale urine has a low specific gravity, a dark line has a high specific gravity.
- Colored urines occur in certain diseases or metabolic disorders, and after the administration of many drugs.

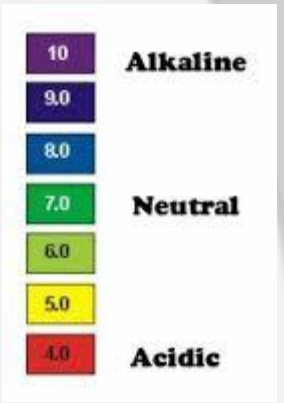
Odor:

- Normally resh urine from most healthy persons has a characteristic aromatic due to the presence of volatile organic acids
- Ammonical odourcould be because of standing due to decomposition of urea.

- The **urine** of patients with diabetes mellitus may have a fruity (acetone) **odor** because of ketosis
- Urine which is infected with Gram-negative organisms often has a distinctive unpleasant smell

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

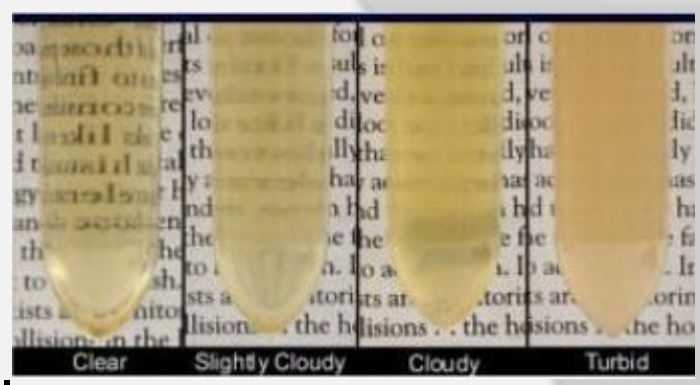
- **Alkaline Urine**:
- A vegetarian diet which causes a tendency to alkalosis
- It may also be grossly increased by
- bacterial infection of the urinary tract

Specific Gravity:

- SG is a measure of the density of the dissolved chemicals in the specimen. As a measurement of specimen density, SG is influenced by both the number of particles present and the size of the particles.

- The normal specific gravity (correctly called relative
- density) of a pooled 24 hour urine sample is between in case of normal hydration and volume usually between 1.010 and 1.025
- Concentrated urine: 1.025–1.030+

Transparency

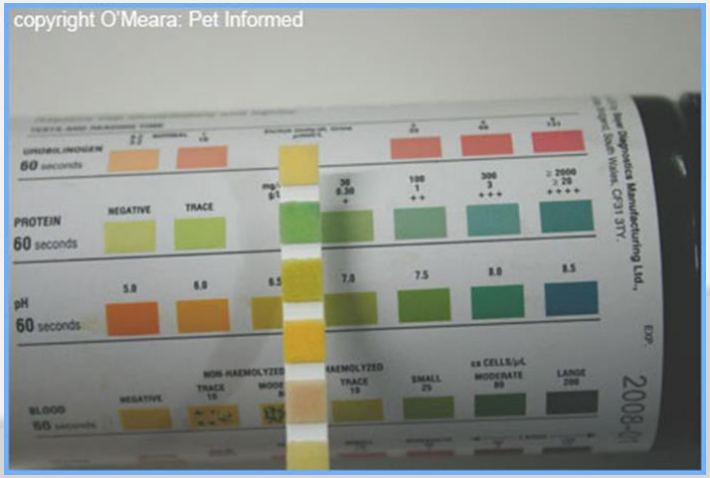


- This is classified as clear and turbid.
- The degree of cloudiness of urine depends on both its pH and its dissolved solids composition.

- (cloudy) urine may be caused by either normal or abnormal processes. Normal conditions giving rise to turbid urine include precipitation of crystals, mucus, or vaginal discharge. Abnormal causes of turbidity include the presence of blood cells, yeast, and bacteria a change in urine pH can cause precipitation, within the bladder, of normal urinary components.

Chemical Examination

- a series of chemical tests is run. Usually, A chemically impregnated dipstick can be used for many of these tests
- For routine chemical analysis of urine there are several brands of chemical test strips (dip sticks) that are commercially available. These urinalysis test strips 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 a ribbon made 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 colour.
- 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, in the latter the colour 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 colours with a colour scale provided by the manufacturer

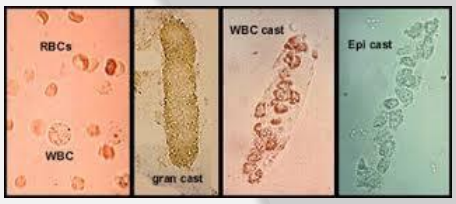
Test strips

- Method:

1) Immerse the test strip **completely** in a well mixed sample of urine for a short time,
2) Extract the test from the container and supporting the edge of the strip over the mouth of the container to remove excess urine.
3) The strip is then left to stand for the time necessary for the reactions to occur (usually 1 to 2 minutes), and
4) The colours that appear are compared against the chromatic scale provided by the manufacturer.

Technique of reagent strips testing

Microscopic examination of Urine sediment



- The urine sediment is centrifuged and examined microscopically for crystals, casts, red blood cells, white bloods cells, and bacteria or yeast.

- Urinary casts

- are cylindrical structures produced by the kidney and present in the urine in certain disease states. They form in the distal convoluted tubule and collecting ducts of nephrons, then dislodge and pass into the urine, where they can be detected by microscopy.

- Urinary casts may be made up of white blood cells, red blood cells, kidney cells, or substances such as protein or fat. The content of a cast can tell your health care provider whether your urine is healthy or abnormal. Normally, healthy people may have a few hyaline casts which constitute from Tamm-horsfall protein

- Crystals:
- Urine contains many dissolved substances (solutes) – waste chemicals that the body needs to eliminate. These solutes can form crystals, solid forms of a particular substance, in the urine if:
- The urine pH is increasingly acidic or basic;
- The concentration of dissolved substances is increased; and
- The urine temperature promotes their formation.
- Crystals are identified by their shape, color, and by the urine pH.
- Crystals may be seen in the urinary sediment of healthy patients
- Uric acid crystals are yellow to orange-brown and may be diamond- or barrel-shaped. Cystine crystals are colorless, have a hexagonal shape, and are present in acidic urine, which is diagnostic of cystinuria.

Practical Part

1-Physical properties of Normal urine:

- By observing and Using test strips

Test	Result
Volume (24 hours)	
Color	
Appearance	
Odour	
pH	
Specific gravity	

2-Chemical Examination :

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

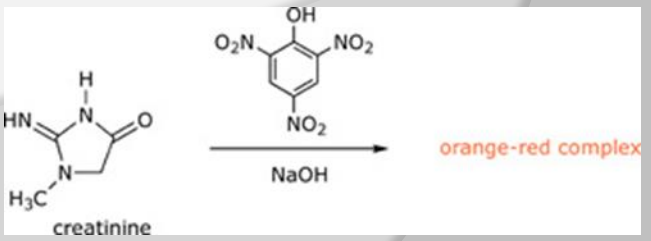
Chemical Examination of normal Urine(organic)

- **Uric acid:**
- Uric acid is the end product of purine metabolism
- To 2 ml of urine add 1 ml of Bendect reagent , then heated
- in a boiling water bath for three minutes . Changes to white precepitate indicates the presence of uric acid

- **Creatinine:**
- Creatinine is spontanusly and irrevesably formed from cretine phosphate in muscle
- Is a metabolic waste product that is not reultlized by the body
- There is a direct relationship between creatinine formation and muscle mass, creatinine production is considered constant from day to day

- Under alkaline conditions creatinine reacts directly with picric ions forming a reddish complex,

- 5 ml of urine add a few drops of saturated solution of picric acid. On rendering the solution alkaline with 2 ml 10% sodium hydroxide solution,
- a deep red color or orange due to creatinine picrate appears. On acidification, with 2N HCl, the color changes to yellow.



Chemical Examination of normal Urine(inorganic)

- **Chloride:**
- Silver chloride is precipitated in the presence of nitric acid and silver nitrate
- Ag(aq) + cl-(aq)---------→ AgCl(s)

- 5 ml of Urine +5 drops of 2N nitric acid+2N silver nitrate solution
- A white precipitate of silver chloride is formed

- **Phosphate:**
- Phosphate ions react with ammonium molybdate to produce a characteristic yellow precipitate, ammonium phosphomolybdate ((NH4)3PO4·12 MoO3).

- 5 ml of urine +4 ml concentrated nitric acid+4 ml of saturated ammonium molybdate
- A yellow crystalline precipitate of ammonium phospho-molybdate appears.

- **Bicarbonate:**
  Sodium bicarbonate (aqueous) reacts with hydrochloric acid (aqueous) to form sodium chloride (aqueous), liquid water, and gaseous carbon dioxide.
- NaHCO3 + HCl(aq) ===> NaCl(aq) + CO2(g) + H2O

- 4 drops of concentrate hydrochloric acid+5 ml of urine.
- A slight effervescence occurs due to CO2 evolution. Test the gas evolved with lime water.

Chemical Examination of normal Urine(inorganic)

- **Sulphate:**
- Its solutions react with sulfate ion to produce a thick white precipitate of barium sulfate.
- $Ba^{2+}_{(aq)} + SO_4^{2-}{}_{(aq)} \rightarrow BaSO_{4(s)}$
- Acidify 10 ml of urine with 1ml dilute hydrochloric acid + 1 ml drops of 5% barium chloride solution
- A white precipitate sulphate is precepitated as of barium sulphate is formed.

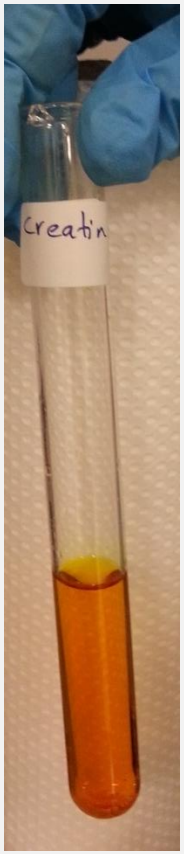
- **Ammonia:**
- **Ammonium** salts produce **ammonia gas** with **sodium hydroxide**. This is an alkaline **gas**. It turns red **litmus** paper blue
- 1 ml of 10% sodium hydroxide solution +5 ml or urine. Boil.
- The evolved ammonia may be detected its occur in confirmed by turning moist red litmus paper blue.

Physical appearance

Test	Result
Volume (24 hours)	800-1500
Color	amer color
Appearance	clear
Odour	aromatic compound
pH	5.5-8
Specific gravity	1.010 and 1.025

Chemical constituent

Test For	reagent	Color
Creatinine	saturated solution of picric acid in alkaline condition	Red-orange color
Uric acid	Bendect 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



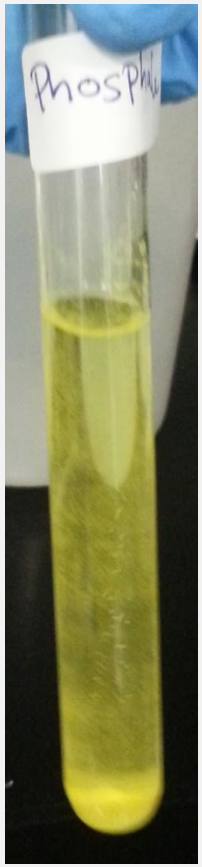
Creatinine

uric acid

White precipitate

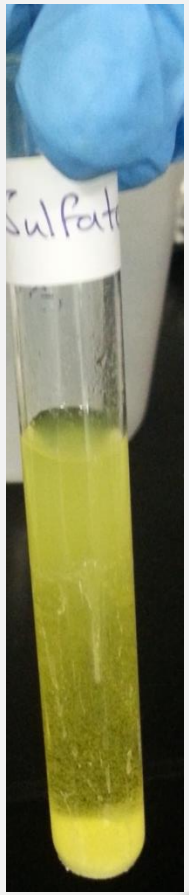
chloride

White precipitate



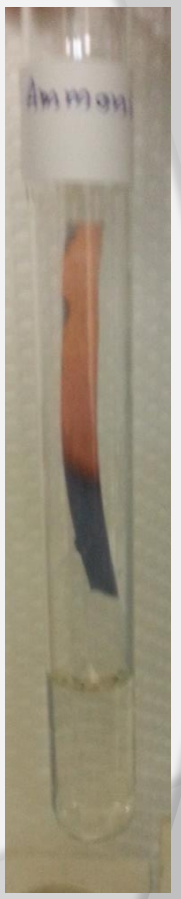
phosphate

yellow precipitate



sulphate

White precipitate



Ammonia