Urine Analysis

Objectives :

At the end of the practical, the student should be able to,

1. Describe how to collect a sample of urine for analysis
2. Interpret the results of macroscopic examination of a sample of urine
3. Identify the following in a sample of urine, under the microscope
    a. RBC
    b. WBC
    c. Epithelial cells
    d. Casts (Hyaline, cellular, granular)
    e. Crystals
4. Explain the physiological significance of the following,
    a. Observation of RBCs, WBCs, Casts or Crystals in a sample of urine
    b. Polyuria, Oliguria and Anuria.
5. Measure the specific gravity of a sample of urine.
6. Describe the technique of measuring urine abnormality.

Introduction:

A urinalysis is a laboratory test. It can help your doctor detect problems that may be shown by your urine. Many illnesses and disorders affect how your body removes waste and toxins. The organs involved in this are your lungs, kidneys, urinary tract, skin, and bladder. Problems with any of these can affect the appearance, concentration, and content of your urine. Your doctor may also order this test if they suspect that you have certain conditions, such as: diabetes, kidney disease, liver disease and urinary tract infection. Your doctor may also want to do a urinalysis if you experience certain symptoms, including: Abdominal pain , back pain ,blood in your urine and painful urination.**Urine** is a fluid obtained from the blood through the renal glomeruli with considerable changes before it is excreted as urine. The first step in urine formation is by ultrafiltration at the glomeruli which is about 170 – 200 L/24 hrs.. There is also active secretion at the renal tubules. Collection of urine specimen depends on the test required either random sample or 24- hours sample. Urine specimen tends to deteriorate unless the correct preservative is added (toluene, chloroform, thymol and formalin) or the specimen is refrigerated throughout the collection period.

Urine samples are usually examined for the main items:

- **Physical examination.**
- **Biochemical examination.**
- **Microscopic examination.**

Urine Analysis

- **Macroscopic**
  - Quantity
  - Quality
    - Colour
    - Appearance
    - Smell
    - Precipitate
- **Microscopic**
  - Cells
  - Casts
  - Crystals
- **Chemical**
  - Sugar
  - Protein
  - Urobilinogen and Urobilin
  - Ph
  - Specific gravity
- Bacteriological
  - Gram stain
  - Culture

Why must the pharmacist study the importance of urine analysis?

-It can detect diseases which pass unnoticed.
For example, D.M, chronic UTI.
- Diagnosis of many renal diseases.As nephrotic, nephritic syndrome, acute renal failure, multiple myeloma
- To give the right medication

Formation of urine :

FILTRATION

It is the first process of urine formation.
20% of fluid pass to the kidney (filtration fraction).
As the blood passes through the glomeruli, much fluids with useful substances (water, Na, glucose) and waste products (urea) will pass in the tubules.

REABSORBTION

It is the passage of fluids from the renal tubules to the peritubular capillaries.
The useful particles reabsorbed from the proximal convoluted tubule till the loop of Henle. Water, 99% of the water filtrate is reabsorbed by passive reabsorbtion.
Glucose, actively reabsorbed in the proximal tubules according to the renal threshold.
Na, actively reabsorbed according to the diet.

Secretion

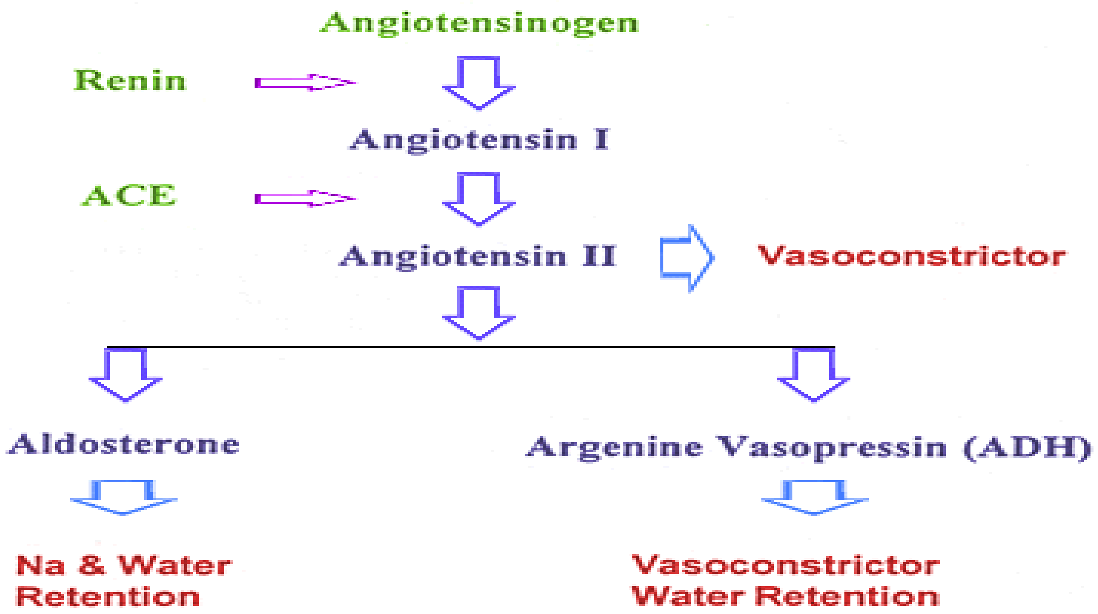
It is the reverse of reabsorption. It is either by active process or by diffusion.

Control Of Urine Excretion:

Antidiuretic Hormone (ADH) . It's a hormone made by the hypothalamus in the brain and stored in the posterior pituitary gland. It tells your kidneys how much water to conserve. ADH constantly regulates and balances the amount of water in your blood. Higher water concentration increases the volume and pressure of your blood.

Aldosterone

aldosterone – secreted by the adrenal cortex, the outer layer of the adrenal gland. The hormone acts mainly in the functional unit of the kidneys to aid in the conservation of sodium, secretion of potassium, water retention and to stabilize blood pressure.



Collection of a sample of urine

Over the course of a 24-hour period, the composition and concentration of urine changes continuously. For this reason, various types of specimens may be collected, including:

- First morning specimen
- Single random specimen
- Timed short-term specimens
- Timed long term specimens: 12 or 24 hours
- Catheterized specimen or specimen from an indwelling catheter
- Double voided specimens (test for sugar and acetone)
- Clean-catch (midstream) specimen for urine culture and cytological analyses

Urine specimens need to be examined within 2 hours. Urine that is left to standing too long becomes alkaline because bacteria begins to split the urea contained in urine into ammonia. Visualization of urine and other tests are inaccurate if the pH of the urine specimen has become highly alkaline. A urine specimen should be refrigerated if it cannot be sent to the laboratory within 2 hours.

Macroscopic appearance of urine

Quantity: **Volume, normally** 700 - 2500 ml/day

↑ Volume - ↑ fluid intake
Consumption of Alcohol
Eating salty foods

↓ Volume - ↓ fluid intake
Dehydration

Anuria is the absence of a urine output and is seen in,

- ✓ Acute renal failure
- ✓ Urinary tract obstruction

Oliguria is a urine output of < 400 ml/day in an adult. Is seen in,

- ✓ Severe dehydration
- ✓ Acute renal failure
- ✓ Acute glomerulonephritis

Polyuria is a urine output of > 2500 ml/day and is seen in,

- ✓ Diabetes Mellitus
- ✓ Diabetes Insipidus
- ✓ Chronic Renal Failure

Quality: **Color**

Characteristic colour of urine is due to the pigments, urochrome and uroerythrin

◊ **Normal color:** is pale yellow or amber yellow (due to urochrome and urobilin pigments).

◊ **Colorless urine:** very diluted urine due to:
-Physiological causes: excessive fluid intake in normal person.
-Pathological causes: uncontrolled diabetes mellitus, diabetes insipidus and chronic renal failure.

◊ **Orange urine: is due to:**

- Ingestion of large amount of carotenoids (vitamin A).
- Concentrated urine (hot weather, high fever, dehydration..etc).

◊ **Yellow - green urine:** is due to bilirubin or biliverdin (jaundice).

◊ **Red urine: is due to:**

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- Some drugs (rifampin for treatment of T.B., carmurit…etc).
- Blood or hemoglobin.

◊ **Dark brown or black urine: is due to:**

- Methemoglobin.
- Melanin (melanoma).
- Malignant malaria (black water fever).

◊ **Smoky urine:** is due to presence of RBCs in acute glomerulonephritis.

Smell (Odor)

a. **Urineferous odor**: normal odor of fresh voided urine (due to presence of aromatic acids).
b. **Fruity odor:** is due to acetone (diabetic ketoacidosis).
c. **Ammoniacal odor:** is due to release of ammonia as a result of the bacterial urease enzyme in the contaminated and long standing exposed urine sample.
d. **Mousy odor:** is due to PKU (Phenylketonuria).
e. **Burnt sugar odor:** is due to maple syrup urine disease.

Deposits:

a. **Normally**, the urine contains no deposits.
b. **Deposits** are mainly due to:

- Crystals, salts or cells.
- Blood clots, necrotic tissues and urinary stones.
- Whitish precipitate seen in heavy proteinuria

Urine Analysis

Chemical examination:

Reaction (pH):

a. Normal range: 4.6 - 7.0 (the average pH is about 6.0).

b. Acidic urine: is due to ketosis (diabetes mellitus & starvation), severe diarrhea, metabolic and respiratory acidosis, excessive ingestion of meat and certain fruits (cranberries).

c. Alkaline urine: is due to:

- Respiratory and metabolic alkalosis.
- Urinary tract infection.
- Vegetarians.

Normal composition of urine:

Urine is a fluid composed of water (95 %) and inorganic and organic solids (5%) that include:

A) **Chief inorganic solids include: -** Sodium - Potassium - Chlorides.
In addition, smaller amounts of calcium, magnesium, sulfate and phosphates, and traces of iron, copper, zinc and iodine.

B) **Chief organic solids are:**
1. Non-protein nitrogen (NPN) compounds.
2. Organic acids
3. Sugars.

Abnormal constituents of urine:

I. Proteinuria:

- Normally less than 200 mg protein is excreted in the urine daily; more than this level leads to a condition called " proteinuria".
- Proteinuria is either glomerular or tubular. **Glomerular** proteinuria is due to increased glomerular permeability leading to filtration of high molecular weight proteins (e.g. glomerulonephritis).**Tubular** proteinuria occurs as a result of decreased reabsorption with normal glomerular permeability leading to excretion of low molecular weight proteins (e.g. chronic nephritis).

The urine should then be proceeded with :

1- **Sulpho-salicylic acid test:** This test for protein is very reliable and does not require heat. In a test tube place about 5 mls of urine and add drops of 20 % sulpho-salicylic acid. The formation of a cloud indicates the presence of protein. The cloud is seen best when looked for against a black background.

2- **Boiling test:** Fill a small test-tube two-thirds full of urine. Add 10 % acetic acid drop by drop and boil, if the cloud disappears it consist of phosphates, if it persist protein is present. Acid should be added drop by drop till no further precipitation of protein occurs.

Clinical finding of proteinuria is classified into three forms:

1. Pre-renal proteinuria:

- **Bence-Jones protein:**
  - This abnormal gamma globulin (light chains only) is synthesized by malignant plasma cells (multiple myeloma).

2. Renal proteinuria:

- After prolonged standing (orthostatic).
- Severe muscular exercise.
- Congestive heart failure, hypertension, fever, stress.

- Gestational (in the 3rd trimester of pregnancy).
- Glomerulonephritis.
- Diabetic nephropathy.

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3. Post-renal proteinuria:

- Lower urinary tract infection, tumors or stones.

II. Glycosuria

presence of detectable amount of any sugar in urine) includes the following:

1. Glucosuria: (presence of detectable amount of glucose in urine).

- **Uncontrolled DM**: The concentration of glucose in the plasma exceeds the renal threshold.
- **Renal glucosuria**: Normal plasma glucose concentration with proximal tubular malfunction leads to decreased renal threshold (gestational diabetes and Fanconi's syndrome).

2. Fructosuria: (Presence of fructose in urine)

- **Alimentary:** due to increased fructose intake.
- **Metabolic:** deficiency of fructokinase or aldolase B enzyme in the liver.

Biochemical tests for diagnosis the sugar in urine:

The presence of a reducing substance in the urine may be detected by means of **Benedict's test** or by the use of **Clinitest tablets**.

Benedict's test: To 5 ml of Benedict's reagent add 8 drops of the urine, boil for 2 minutes and allow to cool. If a reducing substance is present a precipitate will appear, varying from a light green turbidity to a red precipitate. If the reduction is due to glucose the test gives approximately quantitative results as following:

Alight **green** turbidity	=	0.1 to 0.5 % of sugar
A **green** precipitate	=	0.5 to 1.0 % of sugar
A **yellow** precipitate	=	1.0 to 2.0 % of sugar
A **red** precipitate	=	2.0 % of sugar or over

III. Ketonuria: (Presence of ketones "Acetone, acetoacetic acid and β- Hydroxybutyric acid" in urine)

- Diabetic ketoacidosis.
- Glycogen storage diseases.
- Starvation.
- Prolonged vomiting.
- Unbalanced diet: high fat and low carbohydrate diet.

IV. Nitrite:

- Positive nitrite test is significant of bacteruria in urine.

V. Choluria:

a) Bilirubin / bile salts: in cases of

- Hepatocellular damage.
- Obstruction of bile duct: extrahepatic (stone) or intrahepatic (tumors).

b) Urobilinogen:

- Normally, present in trace amounts in urine.
- Markedly increased in:
  - Hemolytic anemia.
  - Hepatocellular damage.

VI. Blood:

a) Hematuria (Presence of detectable amount of blood in urine):

- Acute and chronic glomerulonephritis.
- Local disorders of kidney and genito-urinary tract (trauma, cystitis, renal calculi, tumors …..etc).
- Bleeding disorders (hemophilia).

b) Hemoglobinuria (Presence of hemolysed blood in urine):

- Hemoglobinopathies (sickle cell anemia and thalassemia).
- Transfusion reaction (blood incompatibility).
- Malaria (plasmodium falciparum)

Urine analysis (using dipstick):

Principle:

Dipsticks are plastic strips impregnated with chemical reagents which react with specific substances in the urine to produce **color-coded visual results**. They provide quick determination of pH, protein, glucose, ketones, bilirubin, hemoglobin, nitrite, leukocytes and specific gravity. The depth of color produced relates to the concentration of the substance in urine**.** Color controls are provided against which the actual color produced by the urine sample can be compared.The reaction times of the impregnated chemicals are standardized.

Procedure:

1- Dip the dipstick in the urine sample provided then remove it immediately.
2- Remove the excess urine.
3- Read the color produced within 60 seconds.
4- Match the color changes to the control charts.
5- Give a full report about:
    - Physical examination.
    - Chemical examination.

Microscopic examination of urine :

1 -A sample of well-mixed urine (usually 10-15 ml) is centrifuged in a test tube at relatively low speed (about 2-3,000 r.p.m) for 5-10 minutes until a moderately cohesive button is produced at the bottom of the tube.

2. The supernatant is decanted and a volume of 0.2 to 0.5 ml is left inside the tube.

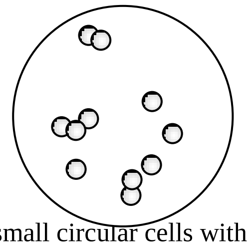
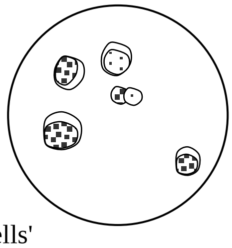
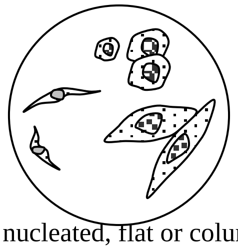
3. The sediment is resuspended in the remaining supernatant by flicking the bottom of the tube several times.

4. A drop of resuspended sediment is poured onto a glass slide and cover-slipped.

5. The sediment is first examined under low power to identify most crystals, casts, squamous cells, and other large objects. Next, examination is carried out at high power to identify crystals, cells, and bacteria

Microscopic appearance of urine

Cells

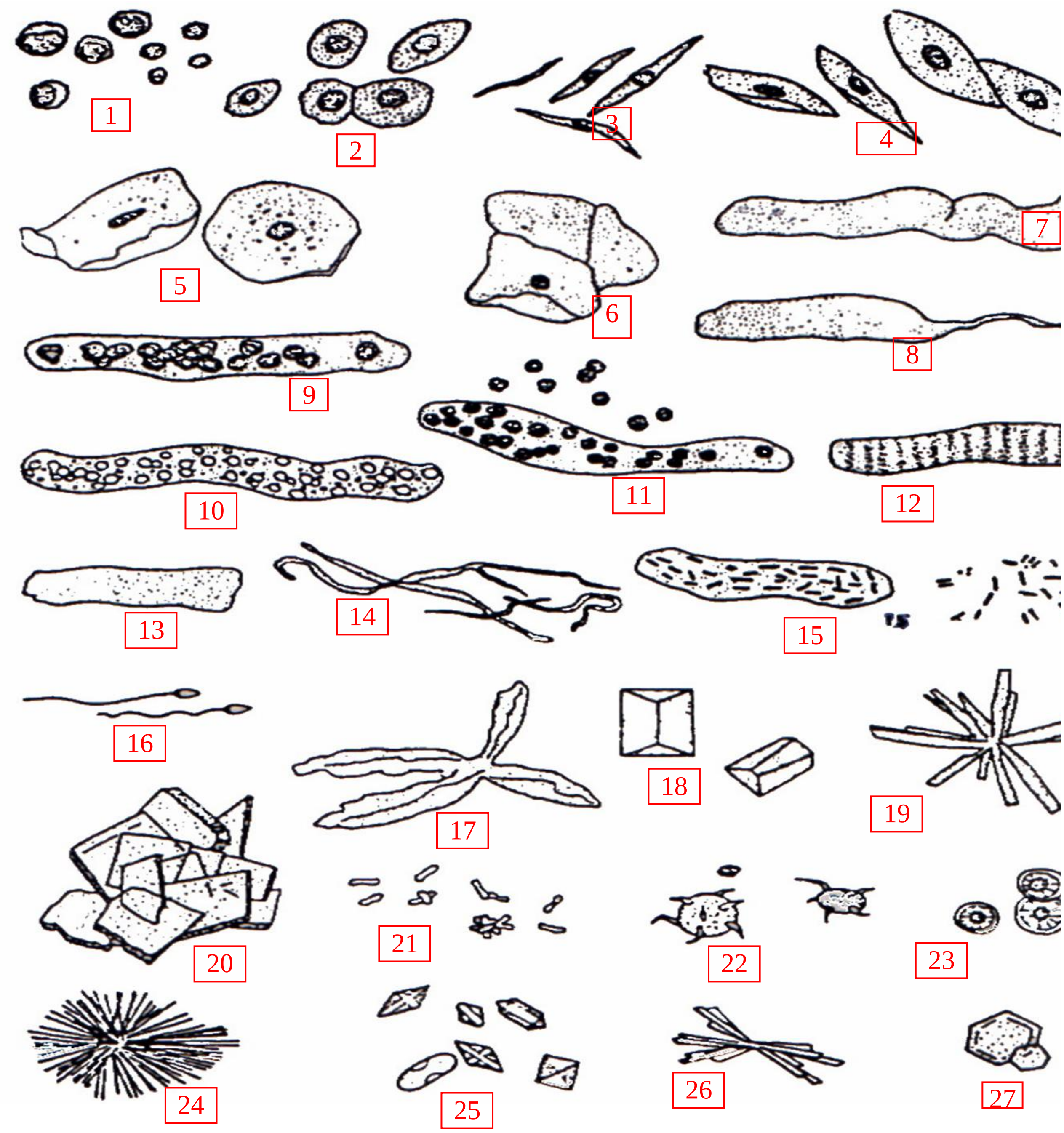
Red Blood Cells	White Blood Cells	Epithelial Cells
 - Are small circular cells with a yellowish center - 2 -3 cells/mm^3 in females - normally no cells in males - Increased amounts seen in, Glomerulonephritis UTI Urinary calculi	 - 'Pus cells' - Larger than RBC - Round shaped with lobed nuclei and a granular cytoplasm - Normally <10cells/mm^3 - Increased amounts in, Cystitis Urethritis Nephritis Prostatitis	 - Are nucleated, flat or columnar cells - Normally, only little amounts seen. - If large amounts, there may be some tubular damage

Casts

- Are cylindrical structures formed by precipitation of muco-proteins (Tamm - Horsefal proteins) in the tubular lumen.
- Hyaline Casts - Pale, transparent and homogenous apperance
- Cellular Casts - Cells are embedded on the hyaline casts, to form RBC, WBC, Epithelial cell casts
- Granular Casts - Disintegrated cells on the cellular casts give the granular Appearance

Crystals

- Are formed by precipitation of chemicals in urine.
- Ammonium, Magnesium, Calcium phosphate crystals (formed in alkaline urine) also known as Triple Phosphate crystals have a "coffin lid" shape.
- Calcium Oxalate crystals are formed in acid urine and have an envelope shape.
- Uric acid crystals maybe normal, but are significantly increased in hyperuricaemia (gout).



1 Leucocyte and pus cell
2,3,4 Polygonal, epithelial cells
5,6 Squamous epithelial cells
7 Hyaline casts
8 Pseudohyaline
9 Epithelial cell cast
10 Erythrocyte cast
11 Leucocyte cast and leucocytes
12 Granular casts (coarse granules)
13 Waxy cast (fine granules)
14 Fibres
15 Bacterial casts and bacteria
16 Spermatozoa
17 ,18 Triple phosphate crystals
19 Calcium hydrogen phosphate
20 Uric acid crystals
21 Calcium carbonate crystals
22 Ammonium urate crystals
23 Leucine crystals
24 Tyrosine crystals
25 Calcium crystals
26 Calcium urate crystals
27 Cysteine crystals

sample collection

Clinical biochemistry deals with the biochemistry laboratory applications to find the cause of a disease as well as the severity of diseases of many organs such as liver, stomach, heart, kidneys, brain as well as the endocrine disorders and related status of acid-base balance of the body. The function of clinical laboratory is to perform qualitative and quantitative analysis on body fluids such as blood (serum or plasma), urine, feces, cerebrospinal fluid (CSF), other body fluids, tissues or calculi.

Sample Collection, such as handling, labeling, processing, a liquoting, storage, and transportation, may affect the results of the study if case sample are handled differently from controls samples, differential misclassification may occur.

The type of sample use in this lab is **BLOOD**

BLOOD Is a liquid connective tissue, suspended in the watery plasma. There are seven types of cells and cell fragments included in blood.
- Red blood cells (RBCs) or erythrocytes
- Platelets or thrombocytes
- Five kinds of white blood cells (WBCs) or leukocytes

The use of skilled technicians and precise procedures when perform phlebotomy are important because painful, prolonged or repeated attempts at venepuncture can cause patient discomfort or injury and result in less than optimum quality or quantity of sample.

Blood can be collected from 3 different sources:

- Venous blood
- Arterial blood
- Capillary blood

Venous blood most commonly required why?

Because most majority of routine tests are performed on venous blood. Blood can be taken directly from the vein. The best sit for venous collection is the deep veins of the ante-cubital fossa.

Requirements for collecting blood sample?

- Sterile Blood needles
- Sterile Syringes
- Plain Vacutainer
- Blood Tubes, Alcohol Prep Pads and Tourniquet.

Spectrophotometry

In chemistry, **spectrophotometry** is the quantitative measurement of the reflection or transmission properties of a material as a function of wavelength. It is more specific than the general term electromagnetic spectroscopy in that spectrophotometry deals with visible light, near-ultraviolet, and near-infrared. Spectrophotometry involves the use of a spectrophotometer. A spectrophotometer is a photometer that can measure intensity as a function of the light source wavelength. Important features of spectrophotometers are spectral bandwidth and linear range absorption or reflectance measurement

In short, the sequence of events in a modern spectrophotometer is as follows:

1. The light source is imaged upon the sample
2. A fraction of the light is transmitted or reflected from the sample
3. The light from the sample is imaged upon the entrance slit of the monochromator.

Blood Collection tubes:

Heparin is an anticoagulant. There are some reports of occasional problems with heparin in PCR assays; studies generally find that there are no major differences in the use of EDTA or heparin

EDTA is an anticoagulant. It works by calcium chelation and is used clinically in hematology studies. It is well suited to DNA-based assays, but has problems for cytogenetic analysis.

Plain tubes is a normal tube with no anticoagulant

Procedure of Serum preparation:

1- Draw blood from patient. Select vacutainer with NO anticoagulant.

2- Allow to stand for 20-30min for clot formation.

3- Centrifuge the sample to speed separation and affect a greater packing of cells. Clot and cells will separate from clean serum and settle to the bottom of the vessel.

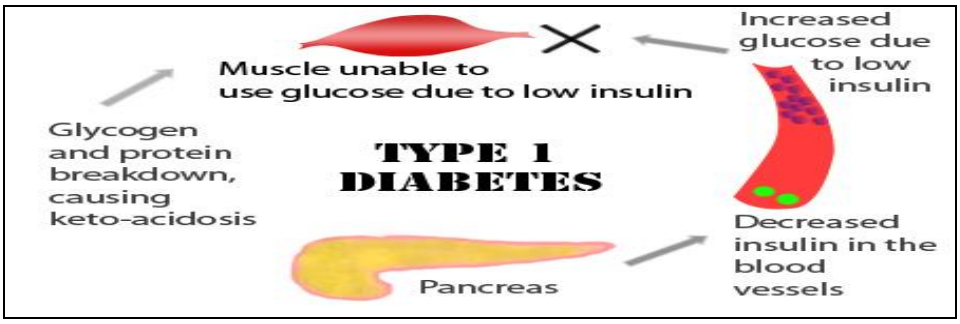
4- The supernatant is the serum which can be now collected by dropper or pipette for testing purposes or stored (-20C to - 80C) for subsequent analysis or use.

(DIABETES MELLITUS)

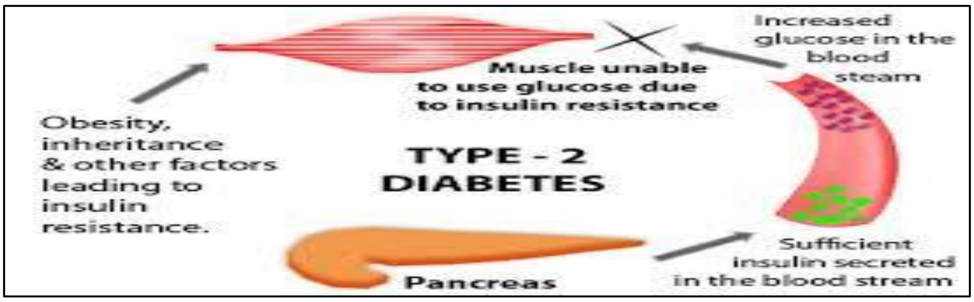
Diabetes mellitus, often simply referred to as diabetes- is a group of metabolic diseases in which a person has high blood sugar, either because the body does not produce enough insulin, or because cells do not respond to the insulin that is produced. This high blood sugar produces the classical symptoms of polyuria (frequent urination), polydipsia (increased thirst) and polyphagia (increased hunger).

There are four main types of diabetes:

*** Type 1 diabetes:** results from the body's failure to produce insulin, and presently requires the person to inject insulin. (Also referred to as insulin-dependent diabetes mellitus, IDDM for short, and juvenile diabetes).



*** Type 2 diabetes:** results from insulin resistance, a condition in which cells fail to use insulin properly, sometimes combined with an absolute insulin deficiency.



*** Gestational diabetes:** is when pregnant women, who have never had diabetes before, have a high blood glucose level during pregnancy. It may precede development of type 2 DM.

*** Pre diabetes:** Other specific types of diabetes result from specific genetic conditions (such as maturity-onset diabetes of youth), surgery, drugs, malnutrition, infections, and other illnesses. Such types of diabetes may account for 1% to 5% of all diagnosed cases of diabetes.
Glucose Testing: Immediate blood sugar; 65-99 mg/dl is the normal range, thus values $\geq$100 indicate elevated glucose.

Normal Blood Glucose Level

Fasting (6 - 8 hr) after meals	***70 - 110 mg/dl***
Random (1 hr) after meals	***110 - 170 mg/dl***
Post prandial (2 hr) after meals	***100 - 150 mg/dl***
Prolonged fasting	***60 - 100 mg/dl***

Types of glucose tests:

- **Random Blood sugar**: (not fasting)
- **Fasting Blood sugar**: (nothing to eat or drink except H2O for 8 hrs)
- **Glucose Tolerance Test**: (Starts fasting, then given sweet drink and measured over time)
- **Hemoglobin A1c**: (Measures glucose control over 3 month)

What is OGTT?

- OGTT (oral glucose tolerance test) is the test to assess the ability of glucose tolerance.
- Glucose tolerance is the ability to dispose of a glucose load effectively.
- In a normal individual, blood glucose level returns to normal levels within 2hrs after ingestion of carbohydrate meal.

Which condition?

OGTT is indicated if a case is highly suspicious of being diabetic but is not diagnosed by random or fasting hyperglycemia.

How to do?

- Fasting 10~14hours.
- A zero time (baseline) blood sample is drawn.
- Drink glucose solution (75g glucose dissolve in 250~300ml H_2O) within 5min.
- Blood is drawn at 30min intervals for at least 2 hours.
- All blood samples are subjected to glucose estimation while urine samples are qualitatively tested for glucose.

1	2	3	4	5
0min	**30min**	**60min**	**90min**	**120min**

The Hemoglobin A1c (HbA1c) Test for Diabetes

The hemoglobin A1c test -- also called HbA1c, glycated hemoglobin test, or glycohemoglobin -- is an important blood test used to determine how well your diabetes is being controlled. Hemoglobin A1c provides an average of your blood sugar control over a six to 12 week period and is used in conjunction with home blood sugar monitoring to make adjustments in your diabetes medicines.

For people without diabetes, the normal range for the hemoglobin A1c test is between 4% and 6%. Because studies have repeatedly shown that out-of-control diabetes results in complications from the disease, the goal for people with diabetes is an hemoglobin A1c less than 7%. The higher the hemoglobin A1c, the higher the risks of developing complications related to diabetes.

Blood Glucose Homeostasis

The balance of insulin and glucagon to maintain blood glucose. Several factors are important for regulating blood glucose level:

- Tissues and organs (Liver and Extra hepatic tissue)

- Hormones
- Kidney
- Gastrointestinal tract

What is hyperglycaemia?

It is the rise of blood glucose level above the normal level.
Symptoms: Dry skin, extreme thirst, needed to urinate often, and slow healing wounds
Causes:
◊ **Deficiency of insulin (Diabetes mellitus)**

◊ **Increase of anti-insulin hormones:**
- Glucocorticoids as in adrenal tumors and Cushing syndrome.
-Thyroxin as in hyperthyroidism.
- Pituitary growth hormone as in acromegaly

What is hypoglycaemia? It is the decrease in blood glucose level below the fasting level.
Symptoms: Sweeting, dizziness, hungry, irritable, headache, weakness and anxious

Management of DM

- Diet and Exercise
- Oral hypoglycaemic therapy
- Insulin Therapy

Lab Practices:

-Collect blood for the test in an appropriate tube.

-Follow the method in the pamphlet.

-Wavelength: . . . . 500 nm
-Cuvette: . . . . 1 cm light path
-Spectrophotometer
-Water bath (Temperature. .. . . 37ºC)

Principle of glucose measurement:

Glucose is determined after enzymatic oxidation by the enzyme glucose oxidase, the formed hydrogen peroxide reacts under the catalysis of peroxidase with phenol and 4-aminophenazone to give the red-violet quinoneimine dye as indicator.

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{GOD}} \text{Gluconic acid} + H_2O_2$$

$$2H_2O_2 + \text{4-aminophenazone} + \text{phenol} \xrightarrow{\text{Peroxidase}} \text{quinoneimine} + 4\,H_2O$$

The intensity of the color formed is proportional to the glucose concentration in the sample.

Procedure:

Wavelength: . . . . . 500 nm
Cuvette: . . . . 1 cm light path
Temperature. .. . . 37ºC / 15-25ºC
Adjust the instrument to zero with distilled water.
Pipette into a cuvette

Standard	*Test*	*Reagents*
	10µl	*Serum*
10 µl		*Standard*
1ml	*1ml*	*Enz&dye reagent*

Mix, incubate for 10 minutes at 20 - 25 C or for 5 minutes at 37C.
Read the absorbance (A) of the samples and standard, against the Blank. The color is stable for at least 30 minutes.

Calculation:

$$\text{Serum glucose} = \frac{\text{Absorption of sample}}{\text{Absorption of Standard}} \times \text{Conc of Sta}$$

Concentration of Standard is 100 mg/dl

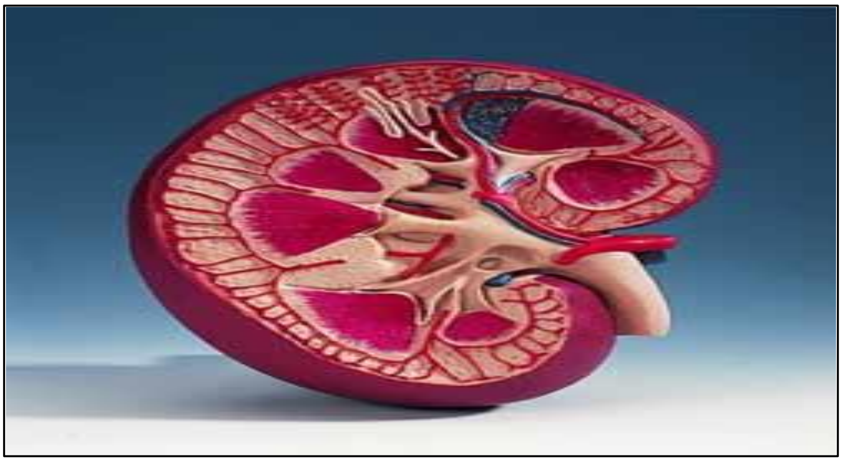
REMEMBER .. TAKE CONTROL OF YOUR LIFE ....DO NOT LET DIABETES CONTROL YOU

(Renal Function Tests)

The kidneys, the body's natural filtration system, perform many vital functions, including removing metabolic waste products from the bloodstream, regulating the body's water balance, and maintaining the pH (acidity/alkalinity) of the body's fluids. Kidney function tests help to determine if the kidneys are performing their tasks adequately. Kidney function tests is a number of clinical laboratory tests that measure the levels of substances normally regulated by the kidneys can help determine the cause and extent of kidney dysfunction. These tests are done on urine samples, as well as on blood samples.

Kidney functions :

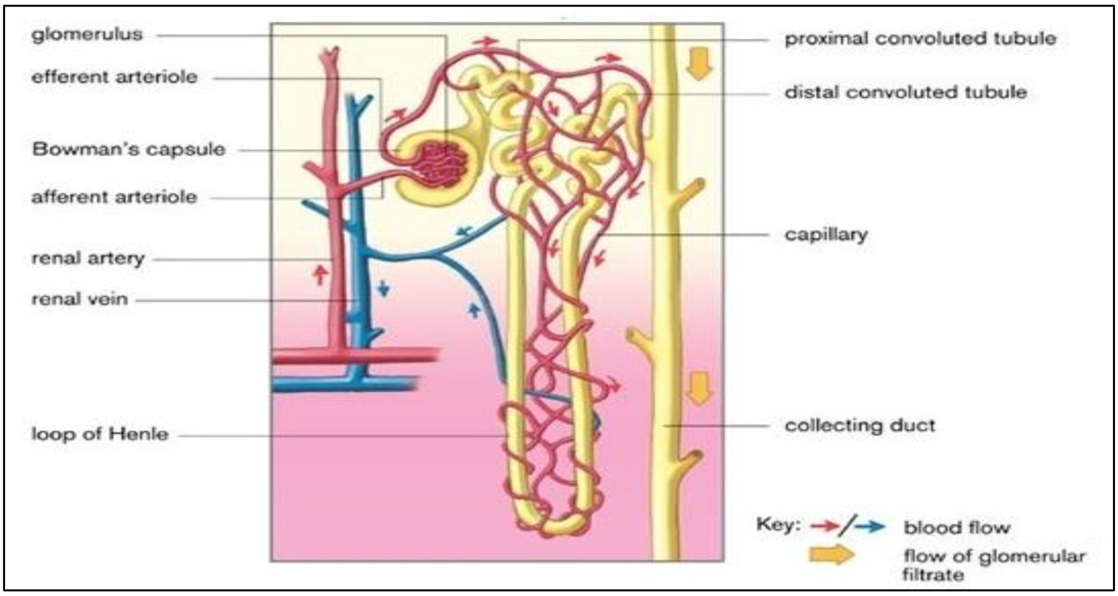
- Regulation of water and electrolyte balance.
- Regulation of acid base balance.
- Regulation of arterial blood pressure.
- Excretion of metabolic waste products and foreign chemicals.
- Hormonal Function : Secretion of erythropoietin



Nephrons and Blood vessels

Functional units :

The nephron is the functional unit of the kidney . Each kidney contains about one million nephrons. The nephron is composed of glomerulus and renal tubules . The nephron performs its function by ultra-filtration at glomerulus and secretion and reabsorption at renal tubules.



Renal diseases :

- Many diseases affect renal function.
- In some, several functions are affected.
- In others, there is selective impairment of glomerular function or one or more tubular function .

Causes of kidney functional disorders

- Pre-renal *e.g.* decreased intravascular volum
- Renal *e.g.* acute tubular necrosis
- Post renal *e.g.* ureteral obstruction

Signs and Symptoms of Renal Failure

- Symptoms of Uraemia (nausea, vomiting, lethargy)
- Disorders of Micturition (frequency, nocturia, dysuria)
- Disorders of Urine volume (polyuria, oliguria, anuria)
- Alterations in urine composition (haematuria, proteinuria, bacteriuria, leukocytouria, calculi)
- Pain
- Oedema (hypoalbuminaemia, salt and water retention)

Why Tests of Renal Function are Important?

- To identify renal dysfunction.
- To diagnose renal disease.
- To monitor disease progress.
- To monitor response to treatment.
- To assess changes in function that may impact on therapy (e.g. Digoxin, chemotherapy).

When should you assess renal function?

- ☐ Older age
- ☐ Family history of Chronic Kidney disease (CKD)
- ☐ Decreased renal mass
- ☐ Low birth weight
- ☐ Diabetes Mellitus (DM)
- ☐ Hypertension (HTN)
- ☐ Autoimmune disease
- ☐ Urinary tract infections (UTI)
- ☐ Nephrolithiasis
- ☐ Obstruction to the lower urinary tract
- ☐ Drug toxicity

Blood tests:

There are several blood tests that can aid in evaluating kidney function. These include:

1-Blood urea nitrogen test (BUN).

2-Creatinine test.

3-Measurement of the blood levels of other elements regulated in part by the kidneys can also be useful in evaluating kidney function. These include sodium, potassium, chloride, bicarbonate, calcium, magnesium, phosphorus, protein, uric acid, and glucose.

1- Blood Urea Nitrogen (BUN)

Urea is the characteristic and most abundant nitrogenous end product of protein catabolism in mammals. It is generated by the liver and excreted by the kidney. Urea filters easily through the glomerulus into the ultra-filtrate. It will diffuse passively into the blood as it passes down the renal tubules. Under conditions of normal flow and normal renal function, about 40% of the filtered urea is reabsorbed; when the flow rate is decreased, the amount passively reabsorbed increases. As with creatinine, the serum concentration of urea nitrogen rises with impaired renal function.

The serum concentration of urea nitrogen is influenced by factors not connected with renal function or urine excretion as it is affected strongly by the degree of protein catabolism. A marked change in dietary protein consumption will be reflected in BUN values. The injection or ingestion of steroids produces a rise in BUN as do stressful situations that cause the adrenal gland to secrete additional cortisol. For these reasons, the measurement of serum creatinine is a better indicator of kidney status than is that of BUN although in many cases, they go up and down simultaneously. The various **prerenal**, **renal**, and **postrenal** factors that affect creatinine also influence the BUN.

Azotemia is a term used to refer to any significant increase in the plasma concentration of non-protein nitrogenous compounds, principally urea and creatinine.

Clinical Significance

The BUN test measures the amount of nitrogen contained in the urea.

High BUN levels can indicate kidney dysfunction, but because blood urea nitrogen is also affected by protein intake and liver function, the test is usually done in conjunction with a blood creatinine, a more specific indicator of kidney function.

A high BUN value can mean:

1-kidney disease, **2-**Blockage of the urinary tract (by a kidney stone or tumor). , **3-** Low blood flow to the kidneys caused by dehydration or heart failure., **4-**Many medicines may cause a high BUN., **5-**A high BUN value may be caused by a high-protein diet, tissue damage (such as from severe burns), or from bleeding in the gastrointestinal tract.

After considering the effects of high protein diet, administration of cortisol-like steroids and stressful situations, increased BUN is seen in the prerenal, renal, and postrenal factors as described for creatinine. **The BUN concentration is low** in late pregnancy when the fetus is growing rapidly and utilizing maternal amino acids, in starvation, and in patients whose diet is grossly deficient in protein.

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Expected Values

Blood urea (20 – 45 mg/dL)

<u>BUN / Creatinine Ratio</u>

- **Pre-renal**

  BUN is more susceptible to non-renal factors

  $\dfrac{\text{Increased BUN}}{\text{Normal Creat}}$

- **Renal**

  Normal Ratio- both BUN and creatinine are proportionally elevated

- **Post-renal**

  Both BUN and Creat. are elevated

  $\dfrac{\text{Increased BUN}}{\text{Increased Creat}}$

2- Creatinine

Creatinine is a non-protein nitrogen waste product formed in muscle. It is excreted in the urine; the amount excreted daily is a function of muscle mass and is not affected by diet, age, sex, or exercise.

Creatinine filters easily into the glomerular filtrate and is not reabsorbed by the tubule. Therefore, any condition that reduces the glomerular filtration rate will result in a lessened excretion from the body, with a consequent rise in the concentration of creatinine in the blood. The serum creatinine is a better indicator of renal function than either that of BUN or uric acid because it is not affected by diet, exercise, or hormones.

Expected Results

Normal range = Males = 0.6 – 1.4 mg /dl
Females = 0.5 – 1.2 mg /dl

Clinical Significance

The serum creatinine is elevated whenever there is a significant reduction in the glomerular filtration rate or when urine elimination is obstructed. The kidney reserve is such, however, that about 50% of kidney function must be lost before a rise in serum creatinine can be detected. Low serum creatinine values have been associated with muscular dystrophy.

A very simple, sensitive chemical method of assessing renal function is the **creatinine clearance test**. This clearance test is an assessment of the glomerular filtration rate, which is an estimate of the amount of plasma that must have flowed through the kidney glomeruli per minute to account for the amount of creatinine appearing in the urine. The test requires complete collection of urine formed during a specified period of time. A serum sample must be obtained from the patient during the urine collection time period. Both the serum and urine creatinine levels are determined in mg/dL. Creatinine clearance is usually performed with 24 hour urine collections, although 12 hour and 4 hour clearances are occasionally ordered.

Normal Values

Males = 105 - 20 mL/min.
Females = 97 - 20 mL/min.

Both serum creatinine and creatinine clearance are used as kidney function tests to :

- Confirm the diagnosis of renal disease.
- Give an idea about the severity of the disease.
- Follow up the treatment.

Creatinine clearance is only recommended in the following conditions:

- Patients with early (minor) renal disease.
- Assessment of possible kidney donors.
- Detection of renal toxicity of some nephrotoxic drugs.

Determination of BUN

Principle

Determination of urea is by the indirect method using the urease-modified Berthelot reaction. Urea is hydrolyzed in the presence of water and urease to produce ammonia and carbon dioxide as in the following equation:

$$\textbf{Urea} + H_2O \xrightarrow{\textbf{urease}} 2\,NH_3 + CO_2$$

In an alkaline medium, the ammonium ions react with the salicylate and hypochlorite to form a green colored dye the absorbance of which is proportional to the urea concentration in the sample.

Procedure:

Working reagent: Mix 1 volume of R1 with 24 volume of R2. The working reagent is stable for 30 days at 2-8 °C

Working reagent	Sample	standard
1 ml	10 µl	-----
1 ml	-----	10 µl

Mix and incubate at 37 °C for 5 minutes. Then add

R3	1ml

Mix and incubate at 37 °C for 5 minutes. Measure the absorbance For the sample and standard at 600 nm.

Calculation:

$$\textbf{Concentration of BUN} = \frac{\textbf{Absorption of sample}}{\textbf{Absorption Standard}} \times \textbf{Conc of Stan}$$

Concentration of Standard is 50 mg/dl

Determination of Creatinine

Kinetic method (Modified Jaffe reaction):

Principle: Creatinine in protein-free filtrate of serum or diluted urine in alkaline solution reacts with picric acid to form a red-orange chromogen the absorbance of which is proportional to the creatinine concentration in the sample.

Procedure:

Working reagent: Mix 1 volume of R1 with1 volume of R2. The working reagent is stable for 30 days at 2-8 °C.

Working reagent	Sample	standard
1 ml	**100 µl**	**-----**
1 ml	**-----**	**100 µl**

Mix gently. Then insert the test tube in to the instrument and start stop watch. Read the absorbance after 30 sec (A1) and after 90 sec (A2). Read at 510 nm.

Calculation

(A2 – A1) sample / (A2 – A1) standard × Conc of Stan

Concentration of Standard is 2 mg/dl

URIC ACID

Uric acid production and metabolism are complex processes involving various factors that regulate hepatic production, as well as renal and gut excretion of this compound. Uric acid is the end product of an exogenous pool of purines and endogenous purine metabolism. The exogenous pool varies significantly with diet, and animal proteins contribute significantly to this purine pool

The serious consequences of abnormal uric acid metabolism depend in part upon the insolubility of uric acid and its sodium monurate salt . The former crystallizes in the kidney and urinary tract while the latter in cartilage and other tissues around the joints in gout .

Plasma uric acid is filtered by the glomerulus's and is subsequently reabsorbed to about 90% by the tubules . Uric acid concentration in serum are greatly affected by extra renal as well as renal factors .

Clinical Significance :

There are some major causes for elevated level of uric acid : Gout, increased nuclear breakdown and renal diseases . Gout is a diseases condition found primarily in males and usually fint diagnosed between the ages of 40-50 yr , patients have pain and inflammation of the joints caused by precipitation of Na urates owing to the high levels of uric acid found in extracellular fluids .

In 25 % - 30 % of these patients hyperuricemia has been shown to be due to overproduction of U. A the plasma UA levels in these patients is usually between 6.5 - 10 mg/ 100ml . .use of their high U.A. levels patients with gout are already highly susceptible development of renal calculi. Another common cause of elevated plasma U.A, levels is in increased breakdown of cell nuclei such as that which occur in patients on chemotherapy for proliferation diseases such as leukemia , lymphomas multiple myeloma or polycythemia.

Elevated levels may also be found after ingestion of a diet rich in purine , or a marked decrease in total dietary intake , resulting in increased tissue breakdown. In general hyperuricemia and hypouricemia are associated the with following clinical disorders.

1 – Hyperuricemia

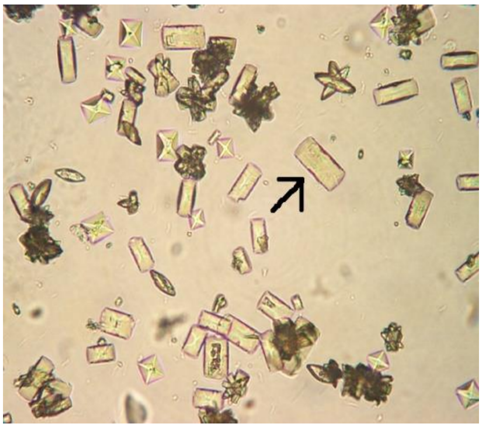
- Acute and chronic Nephritis .
- Gout
- High purine diet
- Urinary obstruction
- Diabetic ketoacidosis
- Leukemia
- Acute infections
- Elevate uric acid levels

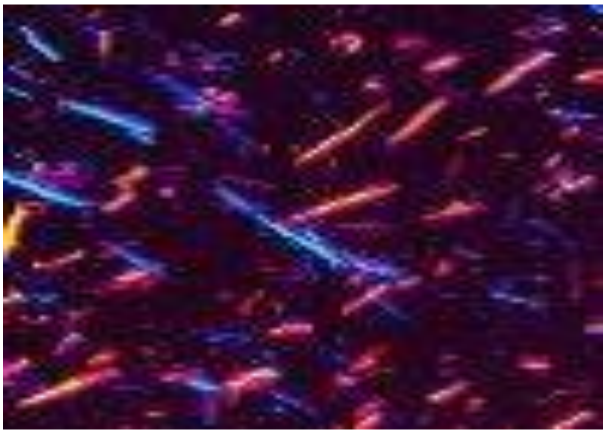
2- Decreased serum uric acid levels are associated with :

- Fanconi syndrome
- Wilson's disease
- Syndrome of inappropriate antidiuretic hormone (SIADH) secretion
- Multiple Sclerosis
- Low purine diet

Gout

Excess serum accumulation of uric acid can lead to a type of arthritis known as gout. This painful condition is the result of needle-like crystals of uric acid precipitating in joints and capillaries. Gout can occur where serum uric acid levels are as low as 6 mg/dL . Man get gout more than women, and at younger ages, the male to female ratio is 9:1. The most common age of onset is from age 40- 60 years.





urate crystal **Uric Acid Crystals Under Polarizing Light Microscopy**

Saturation levels of uric acid in blood may result in one form of kidney stones when the urate crystallizes in the kidney. Very large stones may be detected on x-ray by their displacement of the surrounding kidney tissues. Some patients with gout eventually get uric kidney stones.

A tophus on the elbow of a middle aged man with chronic gout

Principle :

Uric acid is oxidized to allantion and carbon dioxide by a phosphotungstic acid regent in alkaline solution . Phosphotungstic acid is reduced in this reaction to tungsten blue which is measured at 710 nm. Protein have been removed by precipitation with tungstic acid , TCA , phosphotungstic acid and heat coagulation . Other oxidizing agents have included arsenotungstic acid , arsenophosphotungstic acid , arsenomolybdic acid , and potassium ferricyanide . Urea- cyanide was latter used as the alkaline reagent ,but this modification did not required the isolation of U.A. from the filtrate.

Chemically uric acid is 2, 6, 8 trihydroxypurine . It acts like a dibasic acid and can form mono and disodium salts depending on the pH. • Only pH of 5.75 is possible inside body such as in renal tubules. • At this pH, or above it exists as monosodium urate salt. • Thus in plasma, it is mainly as monosodium urate.

Lab Practices:

-Collect blood for the test in an appropriate tube.

-Follow the method in the pamphlet.

-Compare the results to the normal value.

Procedure:

Wavelength: . . . . . 520 nm
Cuvette: . . . . 1 cm light path
Temperature. .. . . 37ºC / 15-25ºC
Adjust the instrument to zero with distilled water.
Pipette into a cuvette

Standard	Test	Reagents
	5 µl	Serum
10 µl		Standard
1ml	1ml	Enz&dye reagent

Mix, and incubate test tube for 5 minute 37ºC. Read the absorbance (A) of the samples and standard, against the Blank

Calculation:

$$\textbf{Concentration of albumin} = \frac{\textbf{Absorption of sample}}{\textbf{Absorption Standard}} \times \textbf{Conc of Stan}$$

Concentration of Standard is 6.0 mg/dl

SERUM PROTEINS

Proteins are the most abundant compounds in your serum (the rest of your blood when you remove all the cells). Amino acids are the building blocks of all proteins. In turn proteins are the building blocks of all cells and body tissues. They are the basic components of enzymes, many hormones, antibodies and clotting agents. Proteins act as transport substances for hormones, vitamins, minerals, lipids and other materials. In addition, proteins help balance the osmotic pressure of the blood and tissue. Osmotic pressure is part of what keeps water inside a particular compartment of your body. Proteins play a major role in maintaining the delicate acid-alkaline balance of your blood. Finally, serum proteins serve as a reserve source of energy for your tissues and muscle when you are not ingesting an adequate amount.

The major measured serum proteins are divided into two groups, albumin and globulins. There are four major types of globulins, each with specific properties and actions. A typical blood panel will provide four different measurements - the total protein, albumin, globulins, and the albumin globulin ratio.

Total Protein

Because the total protein represents the sum of albumin and globulins, it is more important to know which protein fraction is high or low than what is the total protein. Ideally, the total protein will be approximately 7.0 g/dl.

Total protein may be **elevated (** Hyperproteinemia **)** due to:

- Chronic infection (including tuberculosis)
- Liver dysfunction
- Hypersensitivity States
- Hemolysis
- Alcoholism
- Leukemia

Total protein may be **decreased (** Hypoproteinemia) due to:

- Malnutrition and malabsorption (insufficient intake and/or digestion of proteins)
- Liver disease (insufficient production of proteins)
- Diarrhea (loss of protein through the GI tract)
- Severe burns (loss of protein through the skin)
- Hormone Imbalances that favor breakdown of tissue

Serum Albumin

Albumin is synthesized by the liver using dietary protein. Its presence in the plasma creates an osmotic force that maintains fluid volume within the vascular space. A very strong predictor of health; low albumin is a sign of poor health and a predictor of a bad outcome.

Optimal Range: 4.5-5.0 g/100ml

Albumin levels may be **elevated (**Hyperalbuminemia) in:

1) Congestive heart failure
2) Poor protein utilization
3) Congenital

Albumin levels may be **decreased** (Hypoalbuminemia) in:

a. Dehydration
b. Hypothyroidism
c. Malnutrition - Protein deficiency
d. Dilution by excess H_2O (drinking too much water or excess administration of IV fluids)
e. Kidney losses (Nephrotic Syndrome)
f. Liver dysfunction (the body is not synthesizing enough albumin and indicates very poor liver function)

GLOBULINS

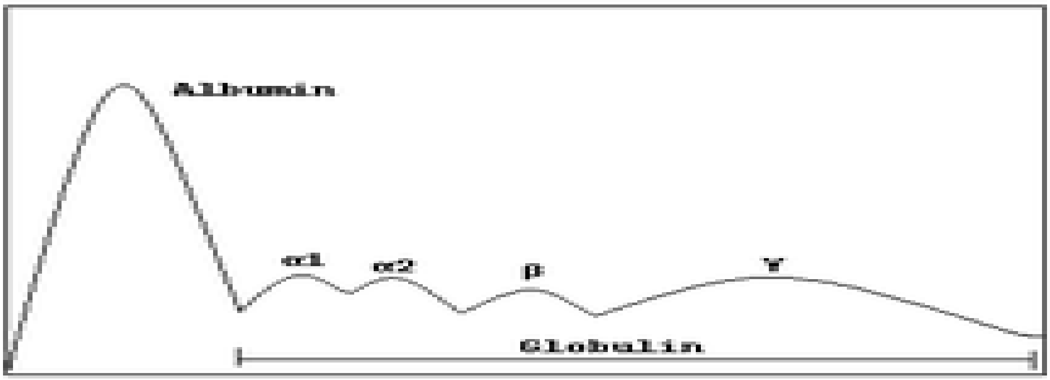
Globulins are proteins that include gamma globulins (antibodies) and a variety of enzymes and carrier/transport proteins. The specific profile of the globulins is determined by **protein electrophoresis** (SPEP), which separates the proteins according to size and charge. There are four major groups that can be identified: **alpha-1 globulins, alpha-2 globulins, beta globulins and gamma globulins**. Once the abnormal group has been identified, further studies can determine the specific protein excess or deficit. Since the gamma fraction usually makes up the largest portion of the globulins, antibody deficiency should always come to mind when the globulin level is low. Antibodies are produced by mature B lymphocytes called plasma cells, while most of the other proteins in the alpha and beta fractions are made in the liver.

Optimal range (gamma Globulin) : 2.3-2.8 g/dL
Optimal range (Alpha Globulin): 0.2-0.3 g/L
Optimal range (Beta Globulin): 0.7-1.0 g/L

The globulin level may be **elevated** in:

- Liver disease (biliary cirrhosis, obstructive jaundice)
- Rheumatoid arthritis
- Multiple myelomas, leukemias,
- Kidney dysfunction (Nephrosis)

Serum protein electrophoresis



Normal serum protein electrophoresis diagram with legend of different zones.

Serum protein electrophoresis (SPEP) is a laboratory test that examines specific proteins in the blood called globulins. Electrophoresis is a laboratory technique in which the blood serum (the fluid portion of the blood after the blood has clotted) is placed on special paper treated with agarose gel and exposed to an electric current to separate the serum protein components into five classifications by size and electrical charge, those being serum albumin, alpha-1 globulins, alpha-2 globulins, beta globulins, and gamma globulins.

Determination of Total serum protein

BIURET METHOD

Principle:

The biuret method depends on the presence of peptide bonds, which react with Cu^{+2} ions in alkaline solutions to form a colored product of unknown composition between the Cu^{+2} and the N atom in the peptide bond of the protein. An analogous reaction occurs between cupric ions and the organic compound biuret; hence the name. The intensity of the color produced is proportional to the number of peptide bonds present in the protein.

Lab Practices:

-Collect blood for the test in an appropriate tube.

-Follow the method in the pamphlet.

-Compare the results to the normal value.

Procedure:

Wavelength: . . . . . 520 nm
Cuvette: . . . . 1 cm light path
Temperature. .. . . 37ºC / 15-25ºC
Adjust the instrument to zero with distilled water.
Pipette into a cuvette

Reagent	sample	Standard
1 ml	**20 µl**	**20**

Mix, and incubate test tube for 5 minute 37ºC. Read the absorbance (A) of the samples and standard, against the Blank

Calculation:

$$\textbf{Concentration of protein} = \frac{\textbf{Absorption of sample}}{\textbf{Absorption Standard}} \times \textbf{Conc of Stan}$$

Concentration of Standard is 7.0 mg/dl

Calcium

Calcium is the most abundant mineral element in the body. It has two key roles. A very small proportion of body calcium plays a vital part in regulating critical functions including nerve impulses, muscle contractions and the activities of enzymes. More than 99% is located in the bones, where it plays an important role in their structure and strength . So critical is calcium's role in metabolic regulation that its concentration in the blood needs to be maintained within a narrow range. If insufficient calcium is obtained from the diet for this purpose , the bones act as a store of calcium from which the element can be withdrawn to keep the blood level constant . In short, we walk about on a vast store of calcium.

Biological functions

- Structural function:

Supporting material in bones. Present as calcium phosphate.

- Signalling function:

Intracellular calcium functions as a second messenger for some hormones.

- Enzymatic function:

Calcium acts as a coenzyme for clotting factors.

Calcium also causes the release of Acetylcholine from Pre-synaptic terminal in the transmission of nerve impulse..

Total calcium is made up of three components : protein bound calcium, ionized calcium and calcium that is complexed with other anions such as phosphate, citrate, bicarbonate and lactate . 99% of calcium in the body is part of bone .

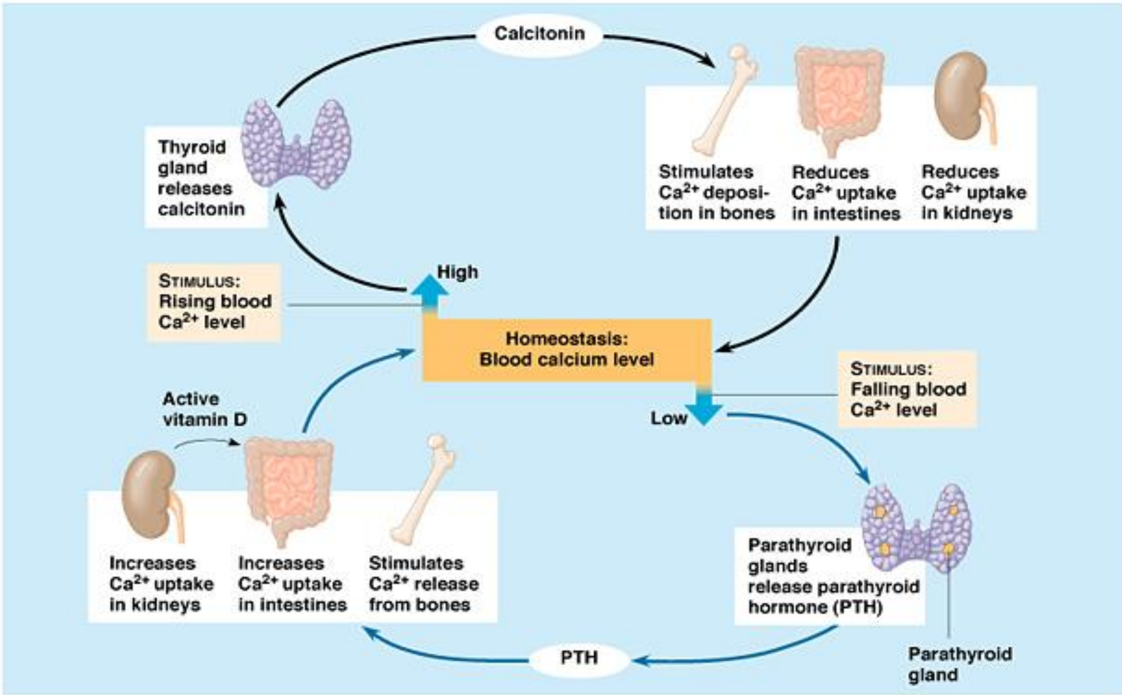
1% in the blood :

45% circulates as free Ca ions

40% bound to albumin

15% bound to anions (**citrate, sulfate, phosphate**) calcium is determined by the amount of ionized calcium, rather than the total calcium. Ionized calcium does not vary with the albumin level, and therefore it is useful to measure the ionized calcium level when the serum albumin is not within normal ranges, or when a calcium disorder is suspected despite a normal total calcium level.

Calcium Regulation



Parathyroid hormone plays the key role. It is secreted from the parathyroid gland when the concentration of Ca++ ions falls below a certain "set point" and acts by controlling the amount of calcium excreted in the urine. It also promotes the metabolism of vitamin D to calcitriol in the kidney and thus indirectly affe c t s intestinal absorption. Parathyroid hormone is also a key regulator of bone remodeling and, hence, the release of calcium from bone.

Vitamin D (Calcitriol) promotes the active absorption of calcium from the small intestine and enhances the reabsorption of calcium by the kidneys. Increases in dietary calcium lead to decreases in the concentration of calcitriol in the plasma, probably through the influence of parathyroid hormone. Calcitriol may also act directly on bone. Together, calcitriol and parathyroid hormone stimulate release of calcium from bone into blood (resorption) and its reabsorption from the kidneys, thus helping maintain the blood calcium concentration.

Calcitonin

Calcitonin is a 32 amino acid peptide hormone synthesized and released by the parafollicular cells (C-cells) of the thyroid. Calcitonin is released in response to high serum calcium levels (and, for unknown reasons, in response to some gastrointestinal .peptides). Like PTH, calcitonin has a short half-life of about 10 minutes

The actions of calcitonin are opposite to those of PTH. In bone, calcitonin inhibits calcium resorption by inhibiting the function of mature osteoclasts and by inhibiting the differentiation of osteoclast precursor cells. In kidney, calcitonin inhibits the reabsorption of both calcium and phosphate

Hypercalcemia Symptoms

- Hypercalcemia of malignancy
- Addison's disease (30-40%)
- Primary hyperparathyroidism
- Bone cancer
- Bone infection (osteomyelitis)

Hypocalcemia Symptoms

- Fainting.
- Heart failure.
- Chest pains.
- Muscle cramps, particularly in the back and legs; may progress to muscle spasm (tetany)
- Difficulty swallowing.
- Voice changes due to spasm of the larynx

Lab Practices:

-Collect blood for the test in an appropriate tube.

-Follow the method in the pamphlet.

-Compare the results to the normal value.

Reagents component of calcium kit : R1 , R2, standard

Procedure:

Wavelength: . . . . . 520 nm
Cuvette: . . . . 1 cm light path
Temperature. .. . . 37ºC / 15-25ºC
Adjust the instrument to zero with distilled water.
Pipette into a cuvette

Working reagent : Mix 0.5 ml R1 with 0.5 ml R2

Reagent	sample	Standard
1 ml working reagent	**10 µl**	**10**

Mix, and let test tube stand for 2 minute at room temperature . Read the absorbance (A) of the samples and standard, against the Blank

Calculation:

$$\textbf{Concentration of Calcium} = \frac{\textbf{Absorption of sample}}{\textbf{Absorption Standard}} \times \textbf{Conc of Stan}$$

Concentration of Standard is 10 mg/dl