Introduction to Clinical Laboratories and sample collection: Lec #1

When a person is ill, diagnosis begins with physical examination by a doctor. It may not possible to diagnose a disease only on the basis of physical examination. There are various diagnostic tests to confirm a suspected diagnosis. The clinical/pathological laboratory tests are extremely useful to find out the causes of disease.

The functional components of the clinical laboratory are:

1) Clinical pathology
2) Hematology
3) Clinical biochemistry
4) Clinical microbiology
5) Serology
6) Blood bank
7) Histology and cytology

Functions:

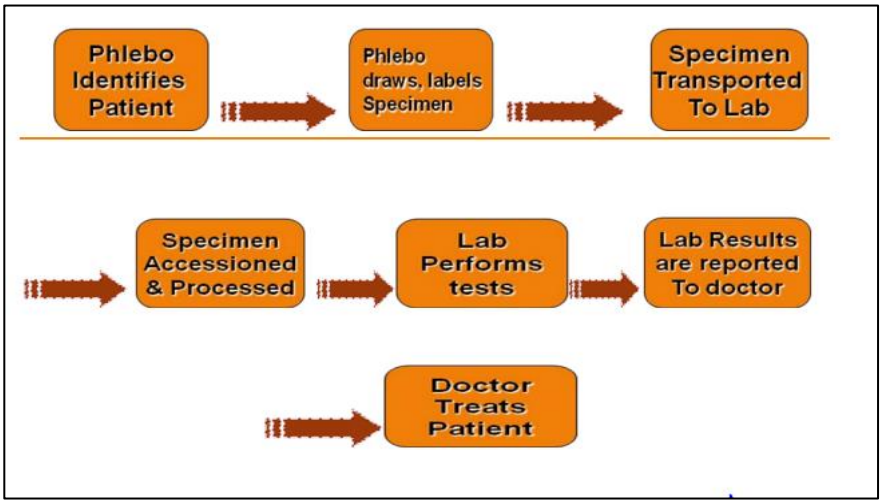
Clinical biochemistry deals with the biochemistry laboratory applications to find the cause of a disease as well as the severity of diseases of many organs such as liver, stomach, heart, kidneys, brain as well as the endocrine disorders and related status of acid-base balance of the body.

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.

- The clinical biochemistry tests, in relation to various clinical conditions can:
1) Reveal the cause of the disease
2) Screen easy diagnosis
3) Suggest effective treatment
4) Assist in monitoring progress of pathological condition
5) Help in assessing response to treatment

Laboratory work flow cycle: The flow cycle includes the entire steps of laboratory test, starting from test ordering by a doctor until reporting the results.



Phlebotomy equipments: The phlebotomist, the technician who collects blood, should be trained to:

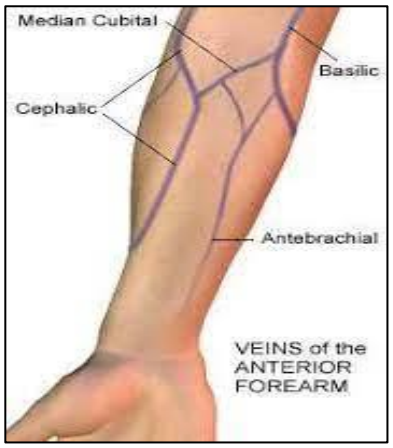
1) Prepare specimen collection material
2) Instruct patient appropriately
3) Collect, preserve and transport specimen carefully
4) Separate serum or plasma properly
5) Maintain proper record of collection
6) Handle the specimen carefully
7) Analyze the specimen accurately
8) Maintain proper record of reports
9) Work with appropriate safety precautions

The phlebotomy equipments: Following material should be readily available in the specimen collection section:

1) Disposable syringes or vacutainer systems
2) Disposable lancets
3) Gauze pads or adsorbent cotton
4) Tourniquet
5) Alcohol swap
6) Waste container

Blood collection:

Selecting vein site: For most venipuncture procedure on adult's vein located in the arm are used. The median cubital vein is the one used for the patient. If the venipuncture of this vein is unsuccessful, one of cephalic or basilic veins may be used. The blood however usually flows more slowly from these veins.



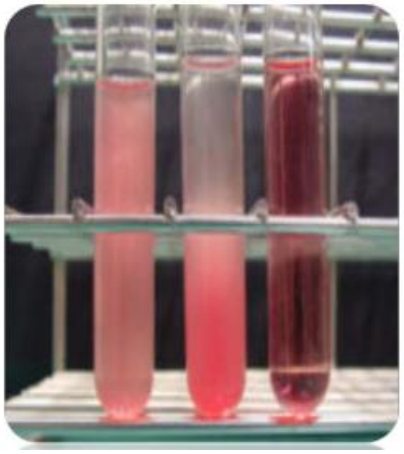
Note: For the determination of blood pH, PCo2, PO2 and bicarbonate, arterial blood is used. It is usually performed by physicians.

Specimen rejection criteria:

1- Specimen improperly labeled or unlabeled
2- Specimen improperly collected or preserved
3- Specimen submitted without properly completed request form
4- If separated plasma or serum is grossly hemolyzd.

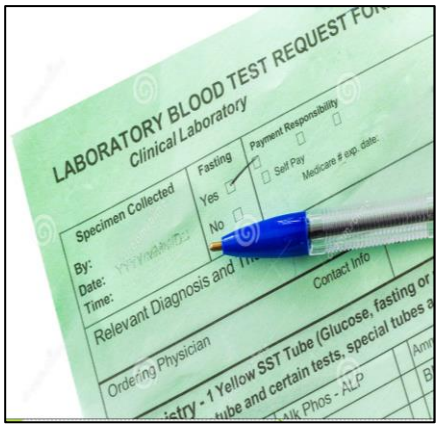
Hemolysis of blood:

Hemolysis means liberation of hemoglobin from RBCs. Due to hemolysis, plasma or serum assumes pink to red color. It is important to avoid hemolysis during sampling, transporting and storage (too hot or too cold) because hemolysis causes changes in measurement of a number of analysis such as:



1- Serum K
2- Serum in.org P.
3- SLDH
4- Acid phosphatase
5- SGOT

Lab request: The order or lab request contains a list of tests to be performed on one or more patient specimen, for example blood or urine.
Each lab has its specific request that contains tests that performed in that lab only i.e. chemistry request, hematology request…etc.



Specimen collection: There are many factors to consider when collecting lab specimens; and prior to diagnostic tests. Preparation of the patient prior to the test or diagnostic measure is vitally important to the results of the test. Many laboratory tests and diagnostic tests do not require any extensive preparation. The amount of the sample needed depends upon many factors. Each lab is different in the amount of blood or other body fluid or tissue required performing the analysis.

Blood collection tubes:

The tubes are covered with a color coded plastic cap. They often include additives that mix with the blood when collected, and the color of the tube's plastic cap indicates which additives that tube contains. The tubes may contain additional substances that preserve the blood for processing in clinical laboratory. Using the wrong tube may therefore make the blood unusable.

-Plasma Separating Tubes (PST): 7 types

LAVENDER (**Purple**)
-It contains EDTA which is a strong anticoagulant.
-It is used mainly for hematology studies.
-It must be inverted several times after collection

LIGHT BLUE
-sodium citrate.
-coagulation (clotting) studies.
-must be completely filled
-must be inverted immediately after filling

GREEN
-sodium or lithium heparin
-for tests requiring whole blood or plasma such as ammonia
-must be inverted several times after collection

Black
-Contains sodium citrate
-Used for ESR
-must be inverted several times after collection

GRAY
-Sodium Fluoride +potassium oxalate.
-It is used for measuring glucose levels.
-must be inverted several times after collection

ROYAL BLUEU
-Heparin or Na EDTA anticoagulants
-Tube is designed to contain no contaminating metals
-Trace element and toxicology studies

YELLOW

-This tube is used for certain reference tests requiring whole blood.
- It contains ACD (acid-citrate-dextrose) as the anticoagulant.
- It is also used for blood cultures.

Serum Separating Tubes (SST):

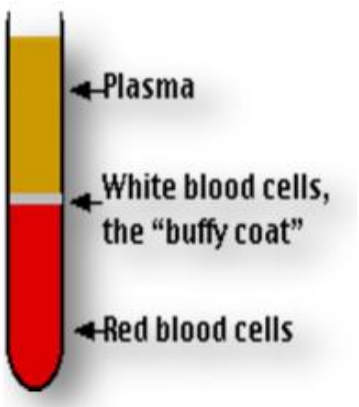
Red (Plain tube)
-contains no additives.
-Tests for antibodies and drugs often require these.

Gold

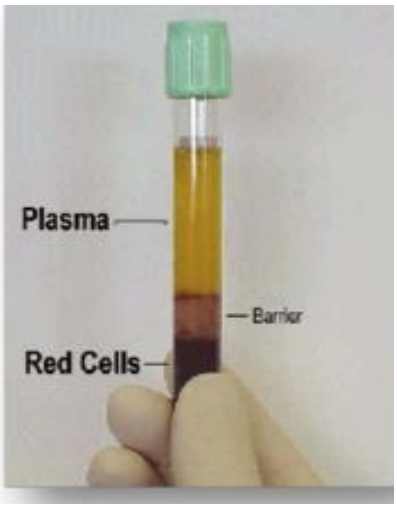
-These contain particles that cause blood to clot quickly, as well as a gel to separate blood cells from serum.

Blood:

Blood is a liquid tissue. Suspended in the watery plasma are seven types of cells and cell fragments.
-Red blood cells(RBCs)
-White blood cells(WBCs)
-Platelets
-Five kinds of Leukocytes
(lymphocytes, onocytes,neutrophils,eosinophils,basophils)
-After centrifugation of blood, the blood separate into three layers(see the figure)



Blood plasma:



-Plasma is the liquid component of blood.
-It is mainly composed of water, blood proteins and inorganic electrolytes.
-Roughly 92% water, mixed with organic and inorganic-substances.
- The most abundant plasma solute is the plasma protein, of which there are three groups: **albumin**, **globulins**, and **fibrinogen**.

Blood clot:

-When a blood sample is left standing without anticoagulant, it forms a coagulum or blood clot.

-One of the normal components of plasma is a soluble plasma protein called **fibrinogen**.
-On standing, this protein will be converted to insoluble substance called fibrin >>>> this occurrence is referred to as blood **coagulation or clotting**.
-The clot contains coagulation proteins, platelets, and entrapped red and white blood cells.

Blood serum:

Serum is the same as plasma except that clotting factors (**such as fibrin**) have been removed.

-For many biochemical laboratory tests, plasma and blood serum can be used interchangeably. Serum resembles plasma in composition **but lacks** the coagulation factors.
- It is obtained by letting a blood specimen clot prior to centrifugation.

Procedure of Plasma Preparation:

1-Draw blood from patient: Select vacutainer with an appropriate anticoagulant.
2- Mix well with anticoagulant.
3- Allow to stand for 10min.
4- Centrifuge the sample to speed separation and affect a greater packing of cells.

5- The supernatant is the plasma which can be now collected for testing purposes or stored (-20C to -80C) for subsequent analysis or use.

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.

Diabetic Profile: Measurement of Blood Glucose…… Lec #2

Diabetes mellitus: is a group of metabolic disorders that requires continuing medical care and patient-self management education to prevent acute complications and to reduce the risk of long term complications.

- It is characterized by **hyperglycemia** and abnormal protein, fat and carbohydrate metabolism due to defects in **insulin secretion**, i.e., inadequate and deficient insulin action on target tissues.

It is classified into 4 clinical classes:

Type I diabetes mellitus (TIDM)

Type 2 diabetes mellitus (TIIDM)

Gestational diabetes mellitus

(GDM) Other specific types due to other causes e.g. Drugs or chemical induced.

Diabetic Profile Tests:

Group of tests that are used to diagnose diabetes and to measure response to treatment, they include:

1-C-peptide…..Differentiates between type I and type II.
2-Blood Glucose.
• FBG (= FBS= PG=FPG)….Fasting blood glucose.
• PP glucose = Post prandial.
• GGT= Glucose tolerance test,
OGGT= Oral glucose tolerance test
3-HbA1c = Glycosylated hemoglobin.
4-Ketones.
5-Microalbuminurea.
6-Insulin.
7-ICA = Islet cell antibodies.

1- C-peptide: Measurement of C-peptide exhibits a number of advantages over insulin measurement.

- It is better indicator of B-cell function than peripheral insulin.
- It doesn't measure the exogenous insulin.
- A C-peptide test **can be done** when diabetes has just been found and it is **not clear** whether Type 1 diabetes or Type 2 diabetes is present. A person whose pancreas does not make any insulin (type 1 diabetes) has a low level of insulin and C-peptide. A person with type 2 diabetes has a normal or high level of C-peptide. Normal value: Fasting …..0.51-2.72ng/ml.

Blood glucose:

- It is a vital component of diabetes management.
- In most cases, significantly elevated fasting glucose levels (>140mg/dl or > 7.77 mmol/L) are, in themselves, usually diagnostic of diabetes. However mild or borderline cases may present with normal FBG values. If diabetes is suspected, GTT can confirm the diagnosis.
- Occasionally, other diseases may produce elevated plasma glucose levels, therefore, a comprehensive history, physical examination and other tests should be carried out to confirm the diagnosis

Types of blood glucose tests:

(1) Fasting blood sugar (FBS) measures blood glucose after fasting for at least 8 hours. It often is the first test done to check for diabetes.

(2) 2-hour postprandial blood sugar (2-hour PP): measures blood glucose exactly 2 hours after eating a meal.

(3) Random blood sugar (RBS): measures blood glucose regardless of when the person last ate. Several random measurements may be taken throughout the day. Random testing is useful because glucose levels in healthy people do not vary widely throughout the day. Blood glucose levels that vary widely may indicate a problem. This test is also called a casual blood glucose test.

(4) Oral glucose tolerance test (OGTT): measures the body's ability to use glucose. It is used mainly to diagnose prediabetes and diabetes. An oral glucose tolerance test is a series of blood glucose measurements taken after you drink a sweet liquid that contains glucose. This test is commonly used to diagnose diabetes that occurs during pregnancy (gestational diabetes). This test is not commonly used to diagnose diabetes in a person.

How to Prepare?

Fasting blood sugar (FBS): For a fasting blood sugar test, the person should not eat or drink anything other than water for at least 8 hours before the blood sample is taken.

Random blood sugar (RBS) No special preparation is required before having a random blood sugar test.

2-hour postprandial blood sugar (2-hour PP) For a 2-hour postprandial test, the subject should eat a meal exactly 2 hours before the blood sample is taken. A home blood sugar test is the most common way to check 2-hour postprandial blood sugar levels.

Oral Glucose tolerance test (OGTT)

• On the day of testing, the following steps will be done:
• A blood sample will be collected when the subject arrives. This is the fasting blood glucose value. It provides a baseline for comparing other glucose values.

• Then a sweet liquid containing a measured amount of glucose will be given to the subject. It is best to drink the liquid quickly. For the standard glucose tolerance test, the subject will drink 75 g to 100 g; pregnant women drink 100 g of glucose.

• Blood samples will be collected at timed intervals of 0.5, 1.1.5, and 2 hours after drinking the glucose.

• The oral glucose tolerance test is not commonly used to diagnose diabetes in people who are not pregnant. Many experts recommend using an oral glucose tolerance tests if the result of a fasting blood glucose test is between 100 mg/dL (5.5 mmol/L) and 126 mg/dL (7.0 mmol/L).

• Glucose tolerance test screening by age 30 is recommend for all women who have polycystic ovary syndrome.

Glycosylated hemoglobin HbA1c:

• Glycosylated hemoglobin is an indicator of the blood glucose concentration over a longer period of time than either a single blood glucose measurement (which reflects the glucose concentration at the time of blood collection)

• A glycohemoglobin test indicates how well diabetes has been controlled in the 2 to 3 months before the test.

• The A1C level is directly related to complications from diabetes: (The lower the A1C level, the lower the risk for complications)

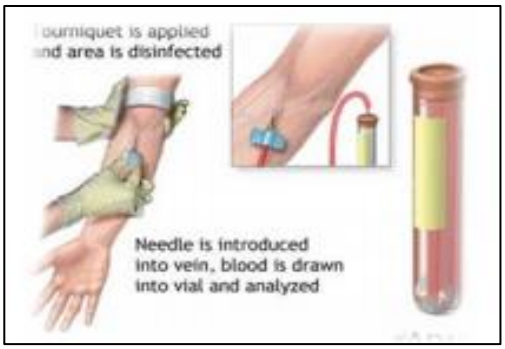
• Normal values vary from lab to lab, depending on the test method used.

Normal Values: Glycohemoglobin A1c: 4.5%-5.7%

Total glycohemoglobin: 5.3%-7.5%

Sample collection:

When whole blood is drawn, allowed to clot and kept centrifuged at room temp, the average decrease in serum glucose is approximately 7% in 1 hour.



It is due to glycolysis by RBCs. The rate of glycolysis after 2 hours increases considerably, so the decrease in serum glucose may be about 50% or more.

- In separated non-hemolyzd serum, the glucose conc. is generally stable for 8 hours at 25C (or up to 72 hours at 4C)
- Glycolysis can be prevented by collecting blood in fluoride tube.
-For HBA1c EDTA tube (whole blood) is used.

Lab Practices:

-Collect blood for FBS in an appropriate tube.
-Follow the method in the pamphlet.
-Compare the results to the normal value.

Principal:

-Glucose oxidase (GOD) catalyses the oxidation of glucose to gluconic acid.

-The formed hydrogen peroxide (HR 2R OR 2R), is detected by a chromogenic oxygen acceptor, phenol-aminophenazone in the presence of peroxidase (POD):

β-D-Glucose + OR 2 R + HR 2R O Gluconic acid + H——→–GODR 2R OR 2 HR 2R OR 2 R + Phenol + Aminophenazone Quinone + H——→–PODR 2R O The intensity of the color formed is proportional to the glucose concentration in the sample.

Procedure:

1. Assay conditions:

Wavelength: . . . . . 505 nm (490-550)

Cuvette: . . . . . 1 cm light path

Temperature. . . . . 37ºC / 15-25ºC

2. Adjust the instrument to zero with distilled water.

3. Pipette into a cuvette

	Blank	Standard	Sample
WR ml	1	1	1
Standard µl	-----	10	-----
Sample µl	-----	-----	10

4. Mix and incubate for 10 min at 37ºC or 15-20 min at room temperature (15-25ºC).

5. Read the absorbance (A) of the samples and standard, against the Blank. The colour is stable for at least 30 minutes.

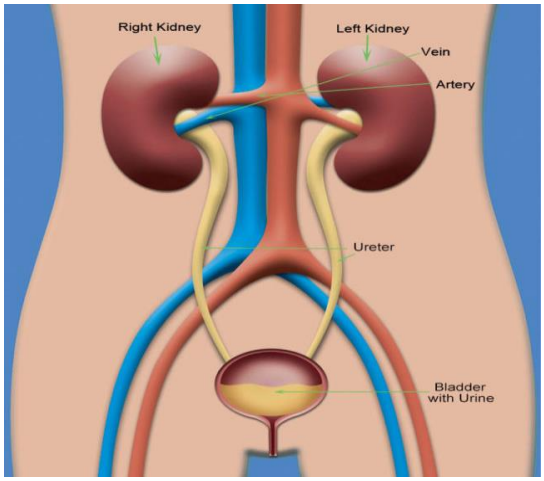
Calculations: A(Sample)/A (Standard) x 100 (Standard conc.) = mg/dL glucose in the sample Conversion factor: mg/dL x 0.0555= mmol/L.

Normal values: Serum or plasma ------60-110mg/dl (3.33-6.10mmol/L)

Linearity: From detection limit of 0.04 mg/dL to linearity limit of 500 mg/dL. If the results obtained were greater than linearity limit, dilute the sample 1/2 with NaCl 9 g/L and multiply the result by 2.

Renal Function Tests, Measurement of Serum BUN…… Lec #3

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.



Why Renal Function Tests 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).

Renal function tests (RFTs) : 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.

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.

I-Measurement of BUN:

- Urea is a by-product of protein metabolism. This waste product is formed in the **liver**, then filtered from the blood and excreted in the urine by the kidneys.
- 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.

Clinical Aspects in the Measurement of BUN:

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.

A low BUN value may be caused by:

1- A diet very low in protein, malnutrition, or severe liver damage.
2-Women and children may have lower BUN levels than men because of how their bodies break down protein.

Lab practices:

Collect blood in an appropriate tube.
-Run BUN test.
-Compare the results to the normal values.

Principal:

Urea reacts with diacetyl-monoxime in hot acidicmedium and in the presence of thiosemicarbazide and ferric ions to form a pink colored compound.

Requirements:

1- Test tubes, 2- 10 ml pipettes, 3- 0.1 ml serologic pipette, 4- Measuring cylinder, 5- Water-bath, 6- Stopwatch and 7- Spectrophotometer

Samples: Serum or heparinized plasma

Preparation of working reagent: It is prepared fresh by mixing one part of reagent 1 + one part of reagent 2 +two parts of reagent 3.
This reagent should be prepared fresh.

Procedure: Pipette in the tubes labeled as follows:

	Test	Standard	Blank
WR(ml)	5	5	5
Serum(µl)	50	-----	-----
Std(µl)	-----	50	------
DW(µl)	-----	-----	50

Mix well and place in boiling water bath for exactly 15 minutes. Cool immediately and after 5 minutes read the absorbance at 520 nm.

Calculations: BUN mg/dl= O.D. test/O.D. standard X20

Linearity: This method is linear up to 50 mg/dl

Normal Values: - Birth-one year: 4-16 mg/dl
- 1-40 years: 7-12 mg/dl -Gradual slight increase occurs over 40
- Panic range: BUN>100 mg/dl

Measurement of Serum Creatinine & Creatinine Clearance:

Creatinine test: This test measures blood levels of creatinine, a by-product of muscle energy metabolism that, like urea, is filtered from the blood by the kidneys and excreted into the urine. Production of creatinine depends on an individual's muscle mass, which usually fluctuates very little. With normal kidney function, then, the amount of creatinine in the blood remains relatively constant and normal. **For this reason,** and because creatinine is affected very little by liver function, an elevated blood creatinine is a more sensitive indication of impaired kidney function than the BUN.

BUN: Creatinine ratio: A BUN test may be done with a blood creatinine test. Blood urea nitrogen (BUN) and creatinine tests can be used together to find the BUN-to creatinine ratio (BUN: creatinine). BUN-to-creatinine ratio 10:1–20:1

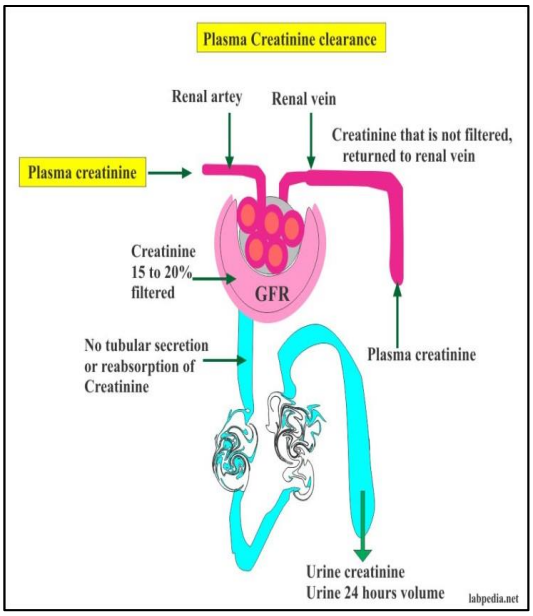
High BUN-to-creatinine ratio: occur with sudden (acute) kidney failure. A blockage in the urinary tract (such as a kidney stone) can cause a high BUN-to-creatinine ratio.

A very high BUN-to-creatinine ratio: may be caused by bleeding in the digestive tract or respiratory tract.

A low BUN-to-creatinine ratio may be caused by a diet low in protein, a severe muscle injury and others.

Other specific test for RFTs:

Creatinine clearance:



In general, creatinine clearance is the removal of creatinine from the body. More accurately, creatinine clearance (ClCr) is the volume of blood plasma?? that is cleared of creatinine per unit time. Clinically, creatinine clearance is a useful measure for estimating the glomerular filtration rate (GFR) of the kidney. The result of this test is an important parameter used in assessing excretory function of the kidneys.

• Measuring serum creatinine is a simple test and it is the most commonly used indicator of renal function. A rise in blood creatinine levels is observed only with marked damage to functioning nephrons. Therefore this test is not suitable for detecting early stage kidney disease.

• A better estimation of kidney function is given by the creatinine clearance test. Creatinine clearance can be accurately calculated using serum creatinine concentration and some or all of the following variables: sex, age, and weight without a 24 hour urine collection

• Some laboratories will calculate the CrC if written on the pathology request form; and, the necessary age, sex, and weight are included in the patient information.

Note: Usually, an adult will need dialysis because symptoms of kidney failure appear at a clearance of less than 10 ml/min. Creatinine clearance has to be measured by urine collection (usually 12 or 24 hours).

It is a more precise estimate of kidney function than serum creatinine since it does not depend on the amount of muscle one

Uric Acid

- Uric acid is formed from the breakdown of nucleic acids and is an end product of purine metabolism.
- Uric acid is transported by the plasma from the liver to the kidney, where it is filtered and where about 70% is excreted in Urine.
- The remainder of uric acid is excreted into the GI tract.

Clinical Significance of Uric Acid

Disease states with increased plasma uric acid

- ✓ Gout
- ✓ Increased catabolism of nucleic acids
- ✓ And renal disease

In Gout increased serum levels of uric acid lead to formation of ***monosodium urate crystals*** around the joints.

Uric acid test is useful to assess for gout and to monitor patients with renal failure

ANALYTIC METHODS for URIC ACID determination:

- **Chemical Method (old) :**
  Phosphotungstic Acid, read the absorbance(Ab) at 700nm (UV). blue Colored product
- **Enzymatic Method: is More specific**
  By using Couple reaction of uricase and Peroxidase. Pink solution (Ab at 500nm spectrophotometric) Pink color solution.

Normal Values: 3.0 – 6.0 mg/dl (Female)
4.0 – 6.0 mg/dl (Male)

Lipid Profile..... Lec #4

Measurement of Serum T-Chol, LDL-C, HDL-C, and TG

What is lipid profile?, Also known as: Lipid Panel; Coronary Risk Panel

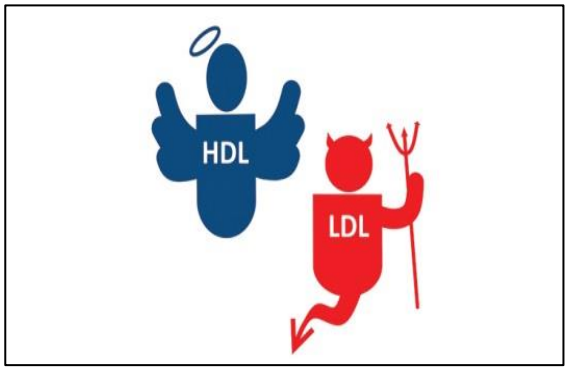
Lipid profile is a group of tests that are often ordered together to determine risk of coronary heart disease. They are tests that have been shown to be good indicators of whether someone is likely to have a heart attack or stroke caused by blockage of blood vessels or hardening of the arteries (atherosclerosis). The lipid profile typically includes:

Total cholesterol

High density lipoprotein cholesterol (HDL-C) - often called good cholesterol

Low density lipoprotein cholesterol (LDL-C) - often called bad cholesterol

Triglycerides



An extended profile may also include:

Very low density lipoprotein cholesterol (VLDL-C)

Non-HDL-C

1- Cholesterol:

Cholesterol is a substance (a steroid) that is essential for life. It forms the membranes for cells in all organs and tissues in the body. It is used to make hormones that are essential for development, growth and reproduction. It forms acids that are needed to absorb nutrients from food. People consume it by eating animal products such as meat, eggs and dairy products. A small amount of the body's cholesterol circulates in the blood in complex particles called lipoproteins.

- Cholesterol is different from most tests in that it is not used to diagnose or monitor a disease but is used to estimate risk of developing a disease specifically heart disease.

Cholesterol levels:

• **Desirable:** Cholesterol below 200 mg/dL is considered desirable and reflects a low risk of heart disease.

• **High Risk:** Cholesterol above 240 mg/dL is considered high risk. The doctor may order a lipid profile (as well as other tests) to try to determine the cause of high cholesterol.

Important note:

1- It is not necessary to fast when you have a cholesterol test. Cholesterol does not change in response to a single meal. Cholesterol does change in response to changes in long term patterns of eating - like changing from a high fat diet to a low fat diet - but it takes several weeks to see changes in blood cholesterol in response to changes in diet.

2- Cholesterol is high during pregnancy. Women should wait at least six weeks after the baby is born to have cholesterol measured.

3- Some drugs that are known to increase cholesterol levels include anabolic steroids, beta blockers, epinephrine, oral contraceptives and vitamin D.

Specimen type: collection and storage:

Serum or plasma can be used. A fasting blood sample is preferred for lipid profile test. However, if cholesterol alone has to be analyzed, a random sample can also be used.

Lab practices:

-Collect blood for serum preparation in an appropriate tube.
-Run lipid profile tests.
-Compare the results to the normal values.

Samples:

Serum: Stability of the sample 7 days at 2-8ºC or freezing at -20ºC will keep samples stable for 3 months.

Procedure:

1. Assay conditions:
Wavelength: . . . . 505 nm (500-550)
Cuvette: . . . . 1 cm light path
Temperature . . . . 37ºC /15-25ºC

2. Adjust the instrument to zero with distilled water.

3. Pipette into a cuvette:

	Blank	standard	Sample
R ml	1	1	1
Standard µl	----	10	-----
Sample µl	----	-----	10

4. Mix and incubate for 5 min at 37ºC or 10 min at 15-25ºC.

5. Read the absorbance (A) of the samples and calibrator, against the Blank. The colour is stable for at least 60 minutes.

Calculations:

A (sample /A (standard) x 200 (Standard conc.) = mg/dL cholesterol in the sample

Conversion factor (cholesterol): mg/dL x 0.0258 = mmol/L.

Conversion factor (TG): mg/dL x 0.0113 = mmol/L.

Reference values:

Cholesterol:

Risk evaluation:

Normal----------less than 200 mg/dl

Borderline-------200-239 mg/dl

High risk----------higher than 240 mg/dl

2- Triglyceride (TG):

This test measures the amount of triglycerides in the blood. Triglycerides are the body's storage form for fat. Most triglycerides are found in adipose (fat) tissue. Some triglycerides circulate in the blood to provide fuel for muscles to work. Extra triglycerides are found in the blood after eating a meal-when fat is being sent from the gut to adipose tissue for storage. The test for triglycerides should be done when you are fasting and no extra triglycerides from a recent meal are present.

What does the test result mean?

A normal level for fasting triglycerides is less than 150 mg/dL. It is unusual to have high triglycerides without also having high cholesterol. When triglycerides are very high (greater than 1000 mg/dL), there is a risk of developing pancreatitis. Treatment to lower triglycerides should be started as soon as possible.

II-Triglyceride:

Men----------40-160 mg/dl

Women----------35-135mg/dl

Measuring range:

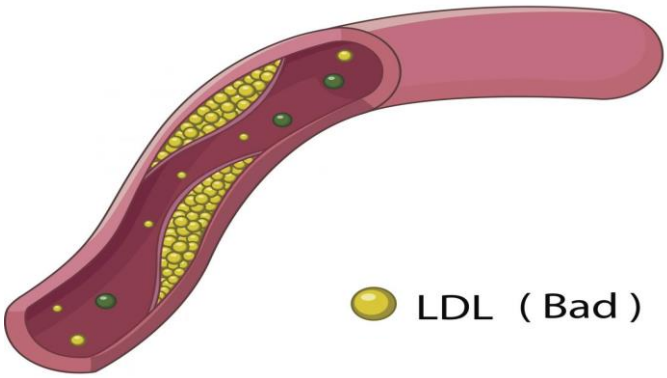
From detection limit 0.46 to linearity limit 600 mg/dL. (Chol)

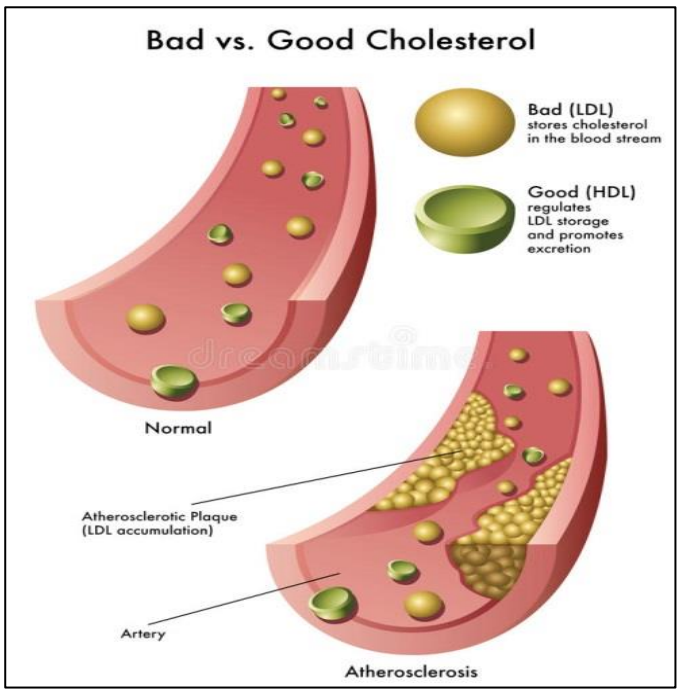
From detection limit 0.7 to linearity limit 1000 mg/dL. (TG)

If the concentration is greater than linearity limit dilute 1/2 the sample with NaCl 9 g/L and multiply the result by 2.

3- LDL-C (BAD CHOLESTEROL):

- It is type of lipoprotein that carries cholesterol in the blood.
- It is considered undesirable because it deposits excess cholesterol in tissues and organs as well as walls of blood vessel and contributes to hardening of the arteries and heart disease.
- Of all the forms of cholesterol in the blood, the LDL-C is considered the most important form in determining risk of heart diseases.





LDL values:

- LDL less than 100 mg/dL if you have heart disease or diabetes.
- LDL less than 130 mg/dL if you have 2 or more risk factors.
- LDL less than 160 mg/dL if you have 0 or 1 risk factor.

How LDL-C level is measured?

1- Direct LDL-C (by using method that measures it directly).

2- Calculated LDL-C (by using equation)

LDL = Total cholesterol – (HDL + TG/5)

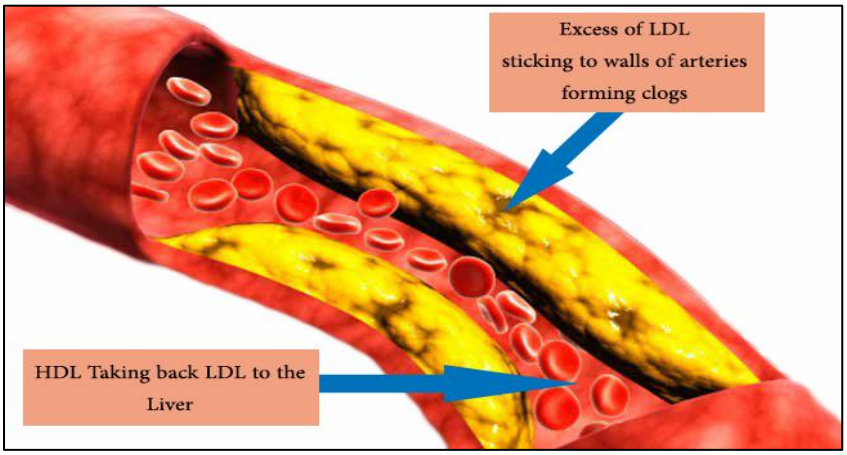
4- HDL-C (GOOD CHOLESTEROL): It is type of lipoprotein that carries cholesterol in the blood.

- ☐ HDL: good cholesterol, carry cholesterol from organs and blood to liver to get rid of it
- ☐ It removes excess cholesterol from tissues (it cleans blood).
- ☐ High levels linked to a reduced risk of heart and blood vessel disease. The higher your HDL level, the better.

HDL values:

• HDL less than 40 mg/dL ..... high risk of heart disease.

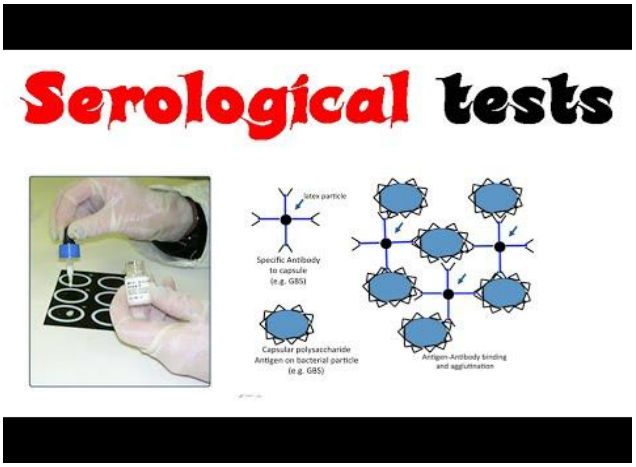
• A good level of HDL is ≥ 60 mg/dL.



Basic principles of immunologic and serologic reactions …… Lec #5

Antibody molecules combine reversibly with antigens to form immune complexes. The detection and measurements of these reactions form the basis of serology, a sub discipline of immunology.

Serology - Is the science of measuring antibody or antigen in body fluids. The immune reaction is the production of antibody (substances) that protect the body against the antigen. There are times, however, when antibodies are not protective (e.g. Hay fever, rash).



Application of serology tests:

Antigen tests: Antigen tests often enable an early diagnosis or presumptive diagnosis of an infectious disease through:-

- Identification of a pathogen that has been isolated by culture
- Identification of pathogens in different samples of the patients, etc

Antibody tests: These tests are used mainly:-

- To diagnose a microbial disease when the pathogen or microbial antigen is not present in routine specimen or if present is not easily isolated and identified by other available techniques.
- To screen donor blood for different infectious diseases
- To monitor the effectiveness of a given treatment by measuring antibody liter
- To diagnose autoimmune disorders, etc.

Immunological Techniques:

Three groups of immunological techniques are used to detect and measure antigen- antibody reaction; these are:

• Primary binding tests

• Secondary binding tests and

• Tertiary binding tests.

Measurement of Ag-Ab reactions: Methods

Many methods are available for the measurement of Ag's and Ab's participating in the Ag-Ab reactions. Measurement may be done in terms of mass or in terms of titer values. The Ab titer of the serum is the highest dilution of the serum which shows an observable reaction with the Ag. The titer value is influenced by the nature and quantity of the Ag and the type and conditions of the test. The two important parameters effecting serological tests are the specificity and the sensitivity. A sensitive test should be able to measure even very minute quantities of Ag or Ab. Specificity refers to the ability of the test to detect the reactions between homologous Ag's and Ab's and not with others. Commercial kits are available in the market to give high quality results.

What is the basis of serological reaction?

1- Antigens will react only with antibodies elicited by itself or by a closely related antigen. Because of?

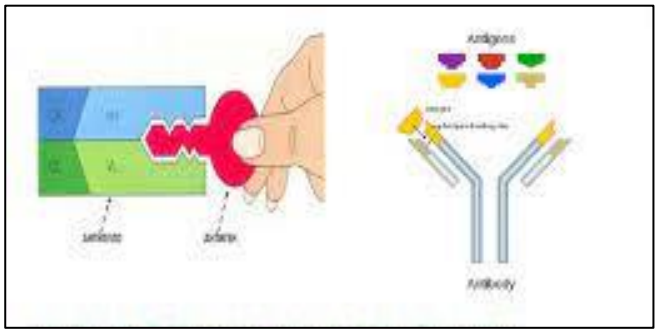
2- Reactions between antigens and antibodies are suitable for identifying one by using the other.

Why antisera containing many different antibodies?

All these bases will depend on the nature of Ag/Ab Reactions which include the following conditions….

Nature of Ag/Ab Reactions:

- **Lock and Key Concept**
- **Non-covalent Bonds**



Hydrogen bonds

Electrostatic bonds

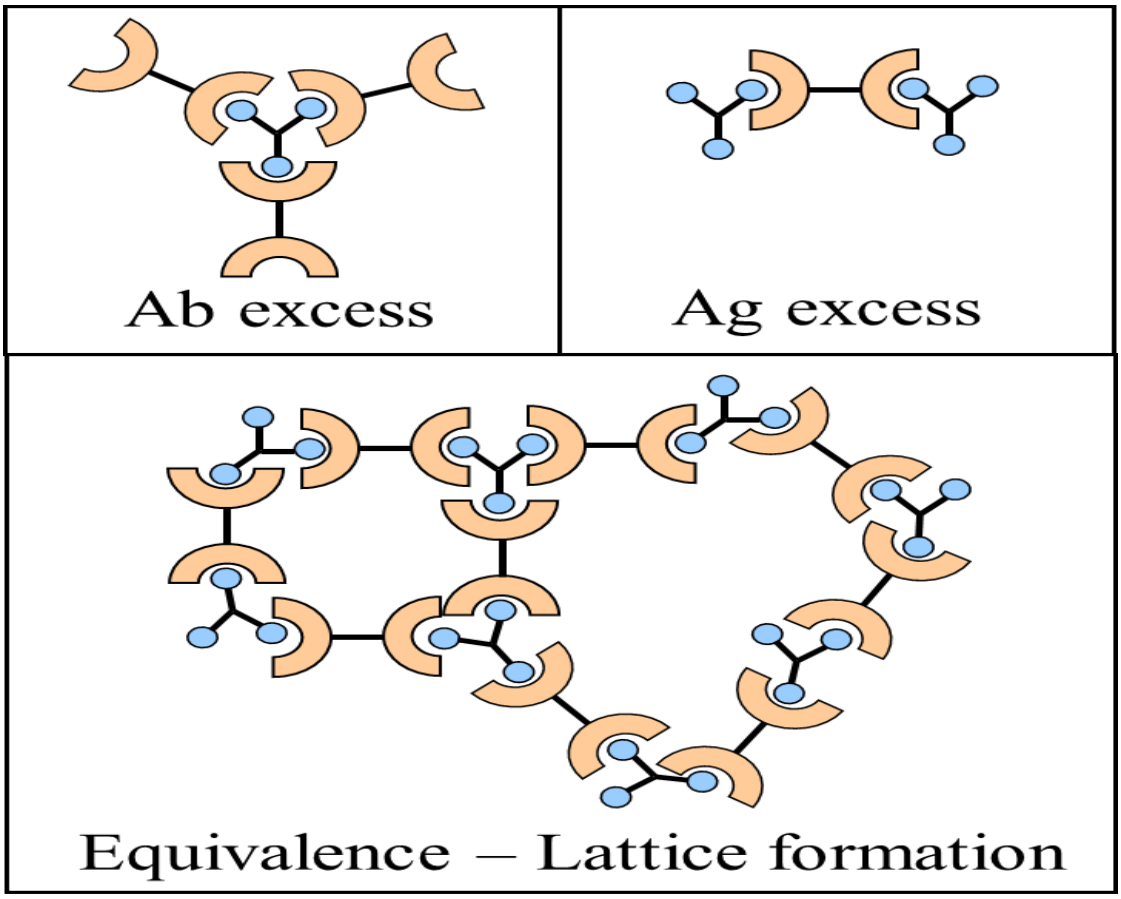
Van der Waal forces

Hydrophobic bonds

- **Multiple Bonds**
- **Reversible**

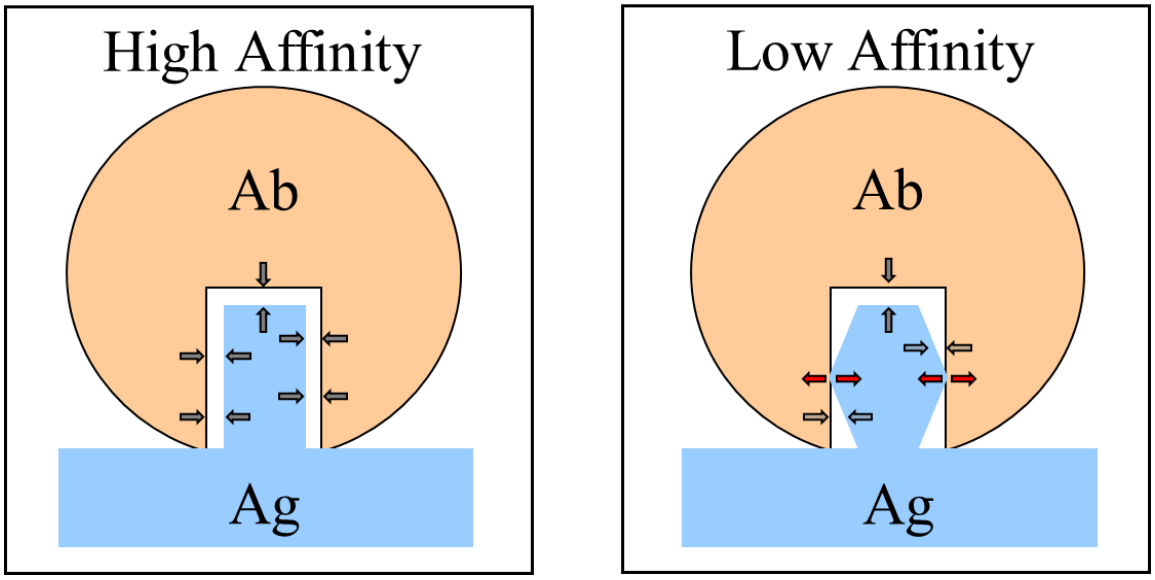
What is the Factors Affecting Measurement of Ag/Ab Reactions??

- Affinity
- Avidity
- Ag: Ab ratio (Specificity)
- Physical form of Ag (Cross Reaction)



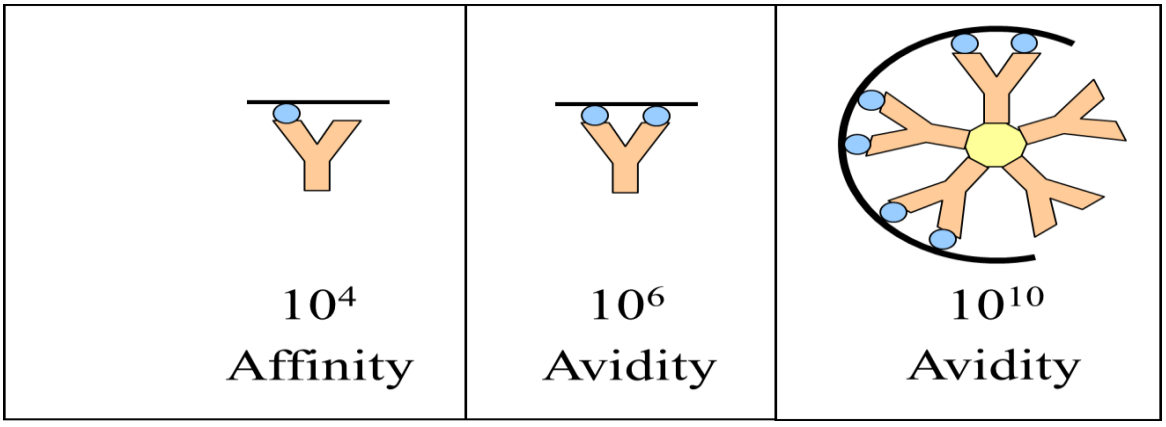
Affinity

- Strength of the reaction between a single antigenic determinant and a single Ab combining site.



Avidity

- The overall strength of binding between an Ag with many determinants and multivalent Abs.

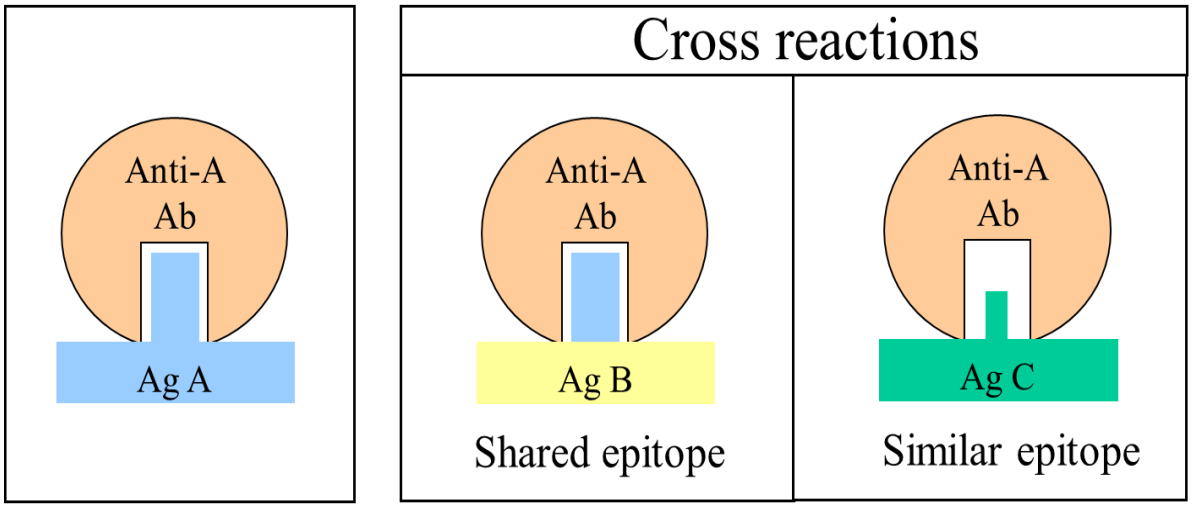


Specificity

- The ability of an individual antibody combining site to react with only one antigenic determinant.
- The ability of a population of antibody molecules to react with only one antigen.

Cross Reactivity

- The ability of an individual Ab combining site to react with more than one antigenic determinant.
- The ability of a population of Ab molecules to react with more than one Ag



Tests Based on Ag/Ab Reactions

- All tests based on Ag/Ab reactions will have to depend on lattice formation or they will have to utilize ways to detect small immune complexes
- All tests based on Ag/Ab reactions can be used to detect either Ag or Ab

Clinical Classification of antigen-antibody interactions:

1. **Primary serological tests: (Marker techniques) e.g.**
   - Enzyme linked immuono sorben assay (ELISA)
   - Immuno flurescent antibody technique (IFAT)
   - Radio immuno assay (RIA)
   - Avidin-Biotin tests
   - Flowcytometry technique (FACS)
2. **Secondary serological tests: e.g.**
   - Agglutination tests
   - Haemoagglutination tests; Complement fixation tests (CFT)
   - Precipitation tests
   - Serum neutralization tests (SNT)
   - Toxin-antitoxin test
3. **Tertiary serological test: e.g.**
   - Determination of the protective value of an anti-serum in an animal.

Agglutination Tests: Agglutination is one of the antigen and specific antibody reactions which takes place when the two are mixed in-vitro in laboratory in the presence of electrolytes at a suitable temperature and pH. Agglutination word comes from the Latin “agglutinare”, meaning "to glue,” it is also clumping of particles. Examples of agglutination in biology are clumping of cells such as bacteria (Widal test) or red blood cells (Blood grouping) in the presence of specific antibody. The antibody binds multiple antigen particles and joins them, creating a large lattice like complex which we can see with naked eye.

Agglutination reaction used for diagnosis of diseases in lab either uses the particulate or soluble antigens. Example of agglutination reaction using particulate antigens is Salmonella typhi bacteria to detect specific antibody in serum from patient suffering from typhoid fever (Widal test). Example of agglutination reaction is latex agglutination and other particle agglutination tests. The soluble antigen is first made particulate by coating it on inert particles like red cells, latex particles, gelatin particles and micro beads. These particles support or carry the soluble antigens to make the reaction visible to naked eye.

Agglutination assays have good sensitivity, do not require sophisticated equipment, are easy to perform, require no wash procedures and are cost effective. The lattice network formed during agglutination reaction can be visualized macroscopically or microscopically as per the directions of the manufacturer.

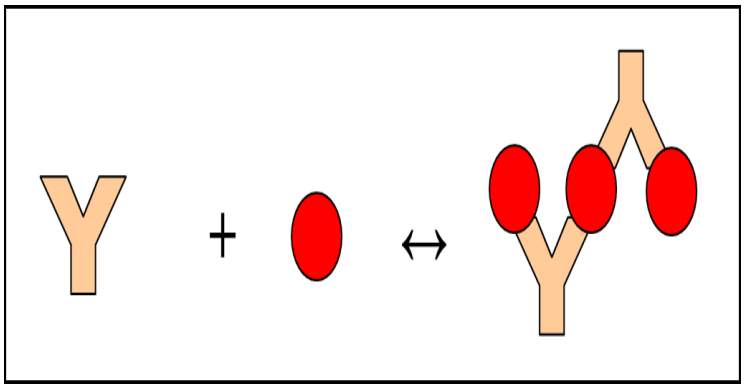
In district laboratories, agglutination tests are frequently used because compared with other serological tests, they are simpler to perform, require no special equipment, and are usually less expensive.

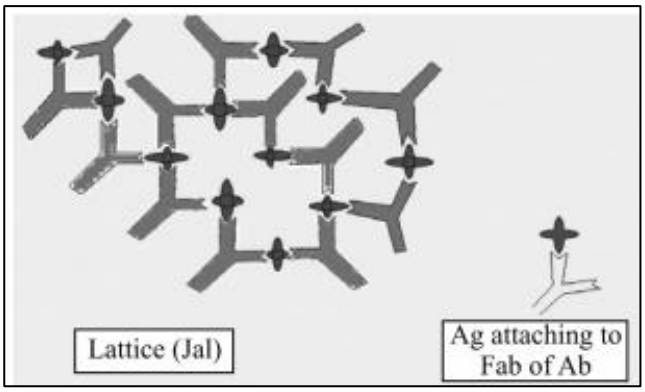
In *this* test, the Ag is particulate (eg, bacteria & red blood cells) and when red cells are used , the reaction is called **hemagglutination** or the Ag is an inert particle (**Latex beads**) coated with an Ag. Ab , because it is divalent or multivalent, cross-links the antigenically multivalent particles and forms a lattice work, and clumping (agglutination) can be seen.

Process of agglutination:

Agglutination is clumping together in suspension of cells bearing the antigen (epitopes)/ antigen bearing microorganisms, or particles in the presence of specific antibodies called “agglutinins”. We can picture a lock and Key concept to understand

the specificity of agglutination reaction. An antibody is a "Y" shaped molecule. The two arms of "Y" are the Fab portion and this has the combining site and is made of the hypervariable regions of the heavy and light chains. The antigenic determinant nestles in a cleft formed by the combining site of the antibody. So the antigenic determinant is the "Key" which fits onto the cleft formed by the "Fab" which is the





lock. If the fit is appropriate then agglutination will happen. This concept is true for all antigen (Ag) antibody (Ab) reactions. The process of agglutination involves two steps. First step is **sensitization** and second is **lattice formation.**

Step-1: Sensitization: It is attachment of specific antibody to corresponding antigen. pH, temperature and time of incubation influence the reaction. **IgM** antibodies react best at 4 to 22 degrees and **IgG** antibodies react best at 37 degrees. Time of incubation can range from 15 to 60 minutes.

Step-2: Lattice formation: Lattice is just like a "Jaal". It is formed by cross linking between sensitized particles. It takes more time than sensitization and we may be able to see the result with naked eyes. **IgM** best at this type of reaction because of large size but **IgG** antibodies may need enhancement.

Methods of enhancing agglutination: These include centrifugation (Bridges distance); treatment with enzymes (Reduces Zeta Potential); colloids (Albumin,

reduces zeta potential) and use of anti- human globulin. Electrokinetic potential in colloidal systems is **termed as Zeta potential**. It is actually the degree of repulsion between adjacent, similarly charged particles in a solution. A high zeta potential confers so it will resist agglutination. So, reducing Zeta potential will favour agglutination.

Methods of agglutination: Agglutination test can be performed using three different techniques. These include: rapid agglutination tests; slow agglutination tests in tubes; slow agglutination tests in micro titration plates.

1- **Tube agglutination tests**

- For laboratory diagnosis of Brucellosis (Rose Bengal test)
- For laboratory diagnosis of Salmonellosis (Widal test)

2- **Slide agglutination tests**

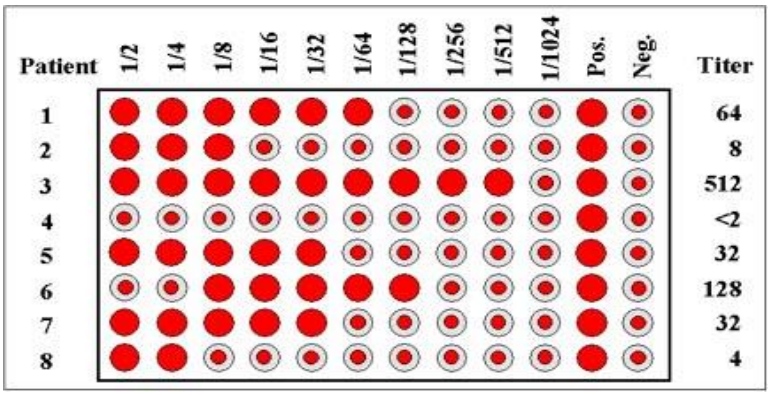
- In determination of ABO blood group
- In determination of Rh factor
- In salmonellosis

3- **Carrier latex particle agglutination tests:**

- Rheumatoid arthritis (RA factor)
- In pregnancy, using agglutination inhibition
- In measurement of serum C-reactive protein (CRP)
- ASO titer

Note: The agglutination tests can be **"Qualitative agglutination test"** - agglutination test used to detect the presence of an antigen or an antibody. The antibody is mixed with the particulate antigen and a positive test is indicated by the agglutination of the particulate antigen. e.g. a patient's red blood cells mixed with antibody to a blood group antigen to determine a person's blood type.

"Quantitative agglutination test" - agglutination tests used to quantitate the level of antibodies to particulate antigens. Serial dilutions of a sample to be tested for antibody are mixed with fixed number of red blood cells or bacteria or other such particulate antigen and the last/highest dilution showing agglutination is the amount of antibody in the sample and is expressed as the titer. The results are reported as the reciprocal of the maximal dilution that gives visible agglutination.



Prozone and Post zone phenomena

False negative antigen antibody reaction, either agglutination or precipitation, can occur if antigen and antibody are not mixed in the right proportions. This can happen if either antibody is in excess (Prozone) or when antigen is in excess (Post zone).

Prozone phenomenon: Some sera when tested un-diluted, do not show agglutination. The same sera when tested after making dilution show a positive agglutination/precipitation reaction. This phenomenon is called "Prozone phenomenon" in which agglutination or precipitation occurs at higher dilution ranges of serum, but is not visible at lower dilutions or when undiluted. Excessive levels of antibody result in false negative reaction as antibody excess results in formation of very small complexes which do not clump to form visible agglutination. Prozone reaction is the probable cause of false-negative result. Prozone reaction can also

result from presence of blocking antibody or to nonspecific inhibitors in serum. When different antigens are located close to each other, the antibodies corresponding to each antigen may block binding by and competing with each other.

Post-zone phenomenon: This refers to the reaction where in excess of antigen results in no lattice formation and a false negative agglutination reaction. Antigen excess is also the probable cause of false-negative antigen-antibody agglutination/precipitation reaction.

The applications of agglutination test in clinical medicine

1- In determine a person's ABO blood group for transfusion.

2- To identify bacterial cultures.

3- To detect the presence relative amount of specific Ab in patients serum.

4- Widely used for rapid diagnosis of several disease such as: -

a- Widal test - Typhoid fever (Salmonellosis)

b- Rose Bengal - Malta fever (Brucellosis)

c- ASOT (Anti - Streptolysin O test).

d- VDRL (Venereal Disease References Lab) for syphilis (*Treponema pallidum*)

What are the agglutination types? There are three types of agglutination:

1- Direct (Active) agglutination: Include

a- Bacterial cells as antigens, e.g.

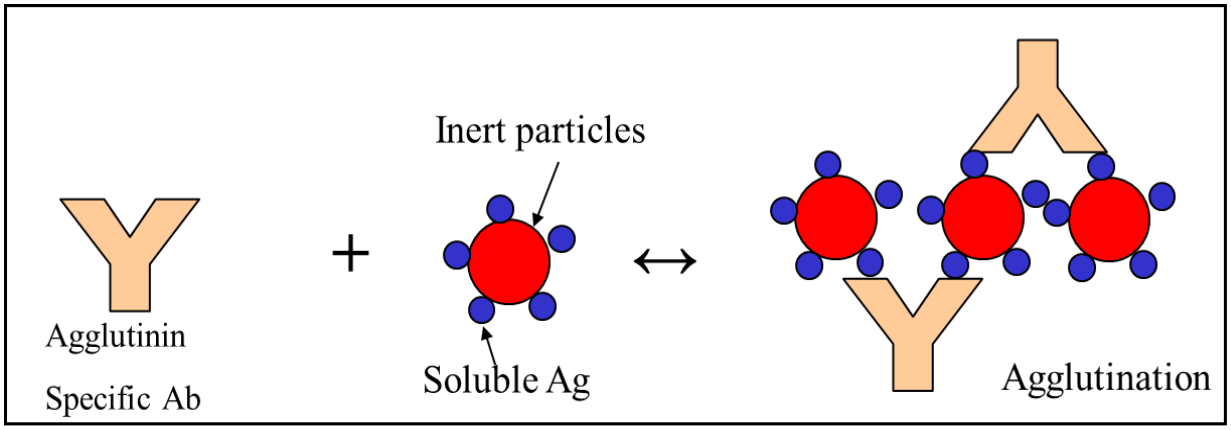
- Widal Test
- Rose Bengal Test

b- R.B.C as antigens; e.g.

- Blood group
- **2- Indirect (Passive) Agglutination:** An inert particle coated with soluble antigens.

Applications

- Measurement of antibodies to soluble antigens



3- Agglutination Inhibition: This test is based on preventing agglutination which leads to positive result. The clinical applications of this test?

This test used to determine if an individual is using certain types of **illegal drugs**, such as (Cocaine or Heroin).

To detect the presence of specific antibody and quantitate the amount of antibody in patient's serum:

One such example is the slide and tube **Widal test**. The agglutinins against '0' (somatic) and 'H' (flagellar) antigens of Salmonella typhi, paratyphi A and paratyphi B are estimated qualitatively (slide test) and quantitatively (Tube test) employing killed suspension of appropriate organisms.

Widal-quantitative tube agglutination test: This test is done to detect antibody against Salmonella typhi, S paratyphi A and S paratyphi B to aid in the diagnosis of enteric fever.

Specimen: Blood sample 3-5 mL is collected in sterile dry screw capped unbreakable tubes and transported to the lab in upright position or stored in refrigerator (2-8°C) in case of delay. Sample can be centrifuged at 3000 rpm for 10 minutes at room temperature to remove particulate matter if required, before performing the test.

Why the titer of widal test is very important factor for patient and the physician? Give us indicator for the following:

1- The severity of the disease.

2- Progression of the disease.

3- Follow up the treatment of patient.

Interpretation: Sera from normal individuals may agglutinate these antigens in dilutions up to 1: 60. Agglutination titers of 1:120 and more are significant and rise in titers or repetition of the test after a few days will confirm the diagnosis of enteric fever. Agglutination titers of 1:240 and above are typically found in cases of enteric fever. However, follow the instructions in the kit insert for interpretation. The specific organism responsible is determined by noting the 'H' agglutinin titre. Persons who have suffered from enteric infections in past or who had received TAB vaccine may show appearance of agglutinins in moderate titer when suffering from other unrelated illness. Such anamnestic appearance of agglutinins can be differentiated from true infection by demonstrating the marked rise/fall in the titer when the test is repeated after 7-10 days. A moderate rise in titer of all three 'H' agglutinins simultaneously against all 'H' antigens is suggestive of recent TAB vaccination.

There are two errors in the lab for doing this test as below:

1- False Negative (Prozone Phenomena): If the antibodies more than antigens.

2- False Positive Results: This occurs under certain causes especially the titer of H-Ag increased for all types of salmonella due to the following:

a- Febrile diseases.

b- Previous Infection

c- Vaccination.

Latex agglutination: As already discussed soluble antigens are coated on inert particles like latex to develop sensitive, specific easy to perform rapid tests termed the latex agglutination tests. Many latex agglutination tests are commercially available and are used to detect either specific antigens or antibody against specific bacteria to diagnose various diseases. Always follow the instructions given in the kit insert to perform the test.

Carrier latex particle agglutination tests (Indirect):

Rheumatoid arthritis (RA factor)

- Rheumatoid arthritis (RA) is a chronic autoimmune disease, it is systemic inflammatory disorder that may affect many tissues and organs, but principally attacks the joints producing an inflammatory synovitis that often progresses to destruction of the articular cartilage of the joints.
- Rheumatoid factor (RF or RhF) is an autoantibody (antibody directed against an organism's own tissues) most relevant in rheumatoid arthritis. It is an antibody against the Fc portion of IgG, which is itself an antibody.
- RF and IgG join to form immune complexes which contribute to the disease process.
- Not all people with rheumatoid arthritis have detectable rheumatoid factor. Those who do not are said to be "seronegative".