

The background features a pattern of red, green, and white geometric shapes. At the top, a white semi-circle is partially visible against a red background. Below this, a white horizontal band contains the text. At the bottom, there are more red and green shapes, including a white semi-circle on the right side.

# ***Serological Tests***

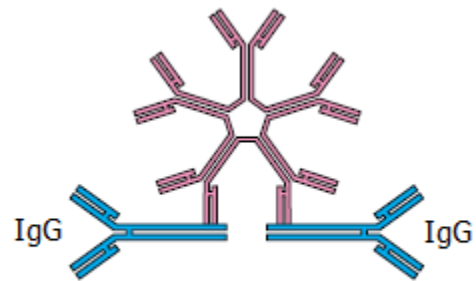
**Rheumatic Factor Test, Rose Bengal Test  
Widal Test, Pregnancy Test  
Lec. 7**

## ***Rheumatic Factor Test***

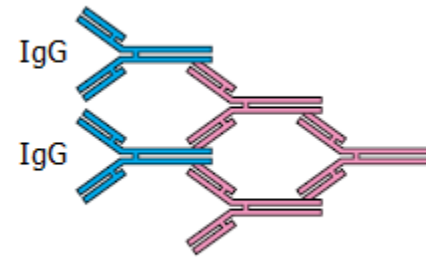
- ❑ Rheumatoid arthritis (RA) is a chronic inflammatory disease affecting primarily the joints and periarticular tissues.
- ❑ As indicated by its name, RF has particular application to diagnosis and monitor rheumatoid arthritis. Rheumatoid factor is detected in 60-80% of cases of diagnosed rheumatoid arthritis.
- ❑ However, it is also detectable sometimes in the serum of patients with Systemic Lupus Erythematosus (SLE) and in certain non-rheumatic conditions. Elevated values may also be observed in normal elderly population.

# Rheumatic Factor Test

- ❖ Rheumatoid Factors (RF) are autoantibodies that react with individuals own immunoglobulin. These antibodies are usually directed against the Fc fragment of the human IgG.



IgM rheumatoid factor

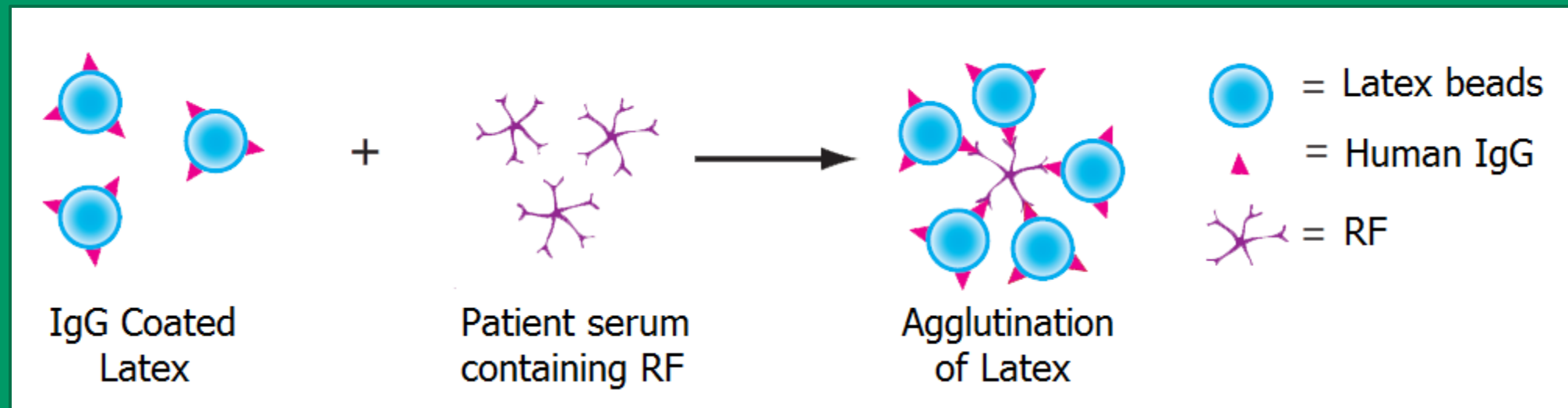


IgG rheumatoid factor

- ❖ RF have been associated with three major immunoglobulin classes: IgM, IgG, and IgA. Of these IgM and IgG are the most common. The formation of immune complex in the joint space leads to the activation of complement and destructive inflammation, causing rheumatoid arthritis.

# Principle of RF test

- ❑ The most commonly used serological method is based on latex agglutination test. As RF is an IgM class of antibody directed against the Fc portion of the IgG molecule, it is detected by its ability to agglutinate the latex particles coated with IgG molecule.
- ❑ Reagent used is a suspension of polystyrene latex particles in glycine-saline buffer with pH:  $8.6 \pm 0.1$ , coated with human gamma globulin.

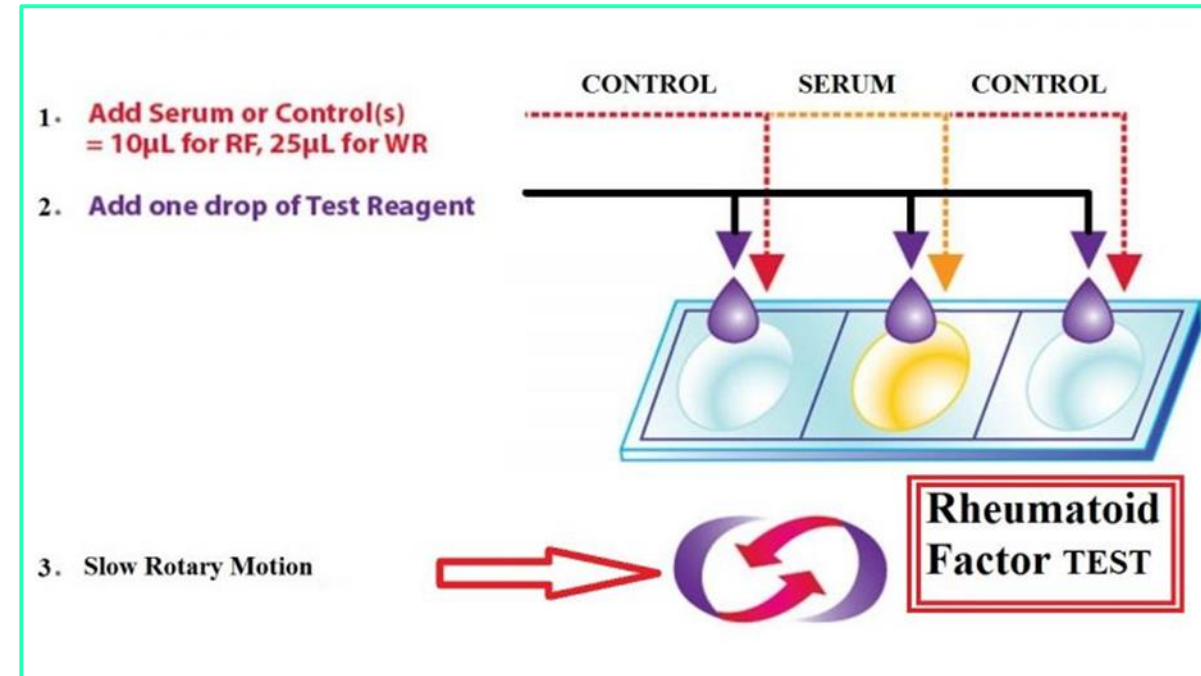




# Qualitative method

## Procedure:

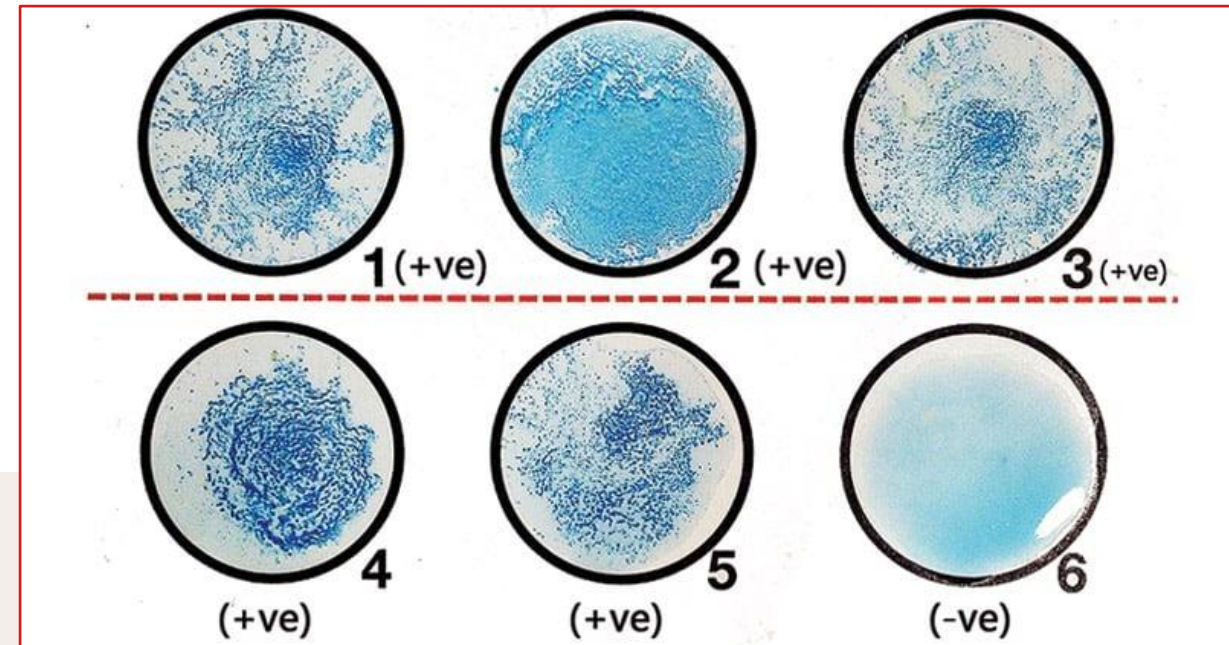
- I. Bring all reagents and specimens to room temperature.
- II. Place one drop of the positive control and 40ul of the patient serum into separate circles on the slide.
- III. Gently add one drop of RF latex reagent on each circle of sample to be tested and control.
- IV. Use separate Applicator sticks/stir sticks to spread reaction mixture over entire area of the particular field.
- V. Tilt the slide back and forth for two minutes in a rotary shaker so that the mixture rotates slowly.
- VI. Observe for agglutination after two minutes under bright artificial light.



# Qualitative method

## Interpretation:

- Agglutination of latex particles is considered a positive reaction, indicating the presence of rheumatoid factor at a significant and detectable level.
- **Positive result:** An agglutination of the latex particles suspension will occur within two minutes, indicating a RF level of more than 18 IU/ml.
- **Negative result:** No agglutination of the latex particles suspension within two minutes.



# Semi-quantitative method

➤ Using isotonic saline prepare serial dilutions of the test sample positive in the qualitative method 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128 and so on as follows :

- I. For each specimen to be tested, add 100  $\mu$ L of 0.9% saline into test tubes numbered 1 to 5.
- II. Add 100  $\mu$ L of specimen onto test tube 1.
- III. Mix the mixture. Avoid formation of bubbles.
- IV. Transfer 100  $\mu$ L of mixed sample from tube 1 to 2.
- V. Repeat this serial dilution procedure in tube 3 to 4, and then 5. Dispose 100  $\mu$ L from test tube 5 after mixing.
- VI. Tubes 1 to 5 now represent a dilution series as follows:

Tube Number:	1	2	3	4	5
Dilution:	1:2	1:4	1:8	1:16	1:32

➤ Perform the qualitative test procedure using each dilution as test specimen.

## Interpretation:

- ✓ The titer is reported as the reciprocal of the highest dilution, which shows a positive test result. Read the titer in the last dilution step with visible agglutination and the approximate concentration of the rheumatoid factor can be determined as follows:

$$\text{RF in IU/mL} = \text{Sensitivity of latex Gamma globulin reagent in IU/mL} \times \text{Titer}$$
$$\text{RF} = 8 * \text{Titer ( a reciprocal highest positive dilution)}$$



# Limitations of RF test

- ✓ The test is not entirely specific & false positive results are seen commonly (3-5%).
- ✓ RF test can become false positive in the following diseases:
  - Chronic liver infections
  - leukemia
  - Infectious mononucleosis
  - systemic lupus erythematosus
  - systemic sclerosis
  - bacterial endocarditis
- ✓ Negative results do not rule out the possibility of RA as up to 20% of rheumatoid arthritis patients remain negative for RF (sero-negative rheumatoid arthritis).
- ✓ False Positive may also be seen in old age, liver disease, chronic lung disease & syphilis.



# Rose Bengal Test

- ❑ The Rose Bengal antigen reaction, or buffered antigen, or brucellosis card test, is a rapid agglutination reaction used widely for identifying **Brucellosis** antibodies in sera.
- ❑ The antibodies IgG & IgM appear in 7-10 days after clinical infection.
- ❑ As the disease progresses, IgM antibodies decline while IgG antibodies persist or even increase.
- ❑ Brucella's IgG antibodies have long life span (1-2 yr.)
- ❑ **Prozone phenomena** is the presence of high antibody titer that lead to Ag block and hence, false negative results are obtained. Dilution will resolve this problem.
- ❑ This phenomena appears obviously in Brucella serology test.





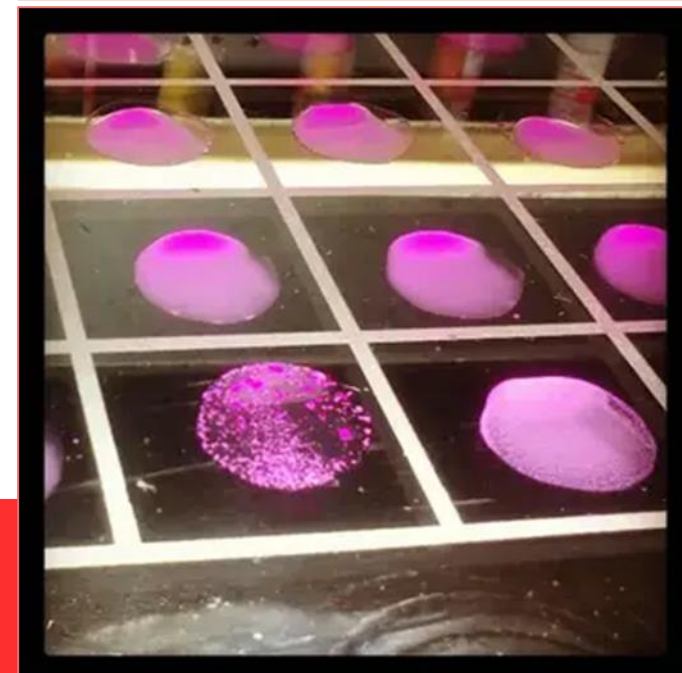
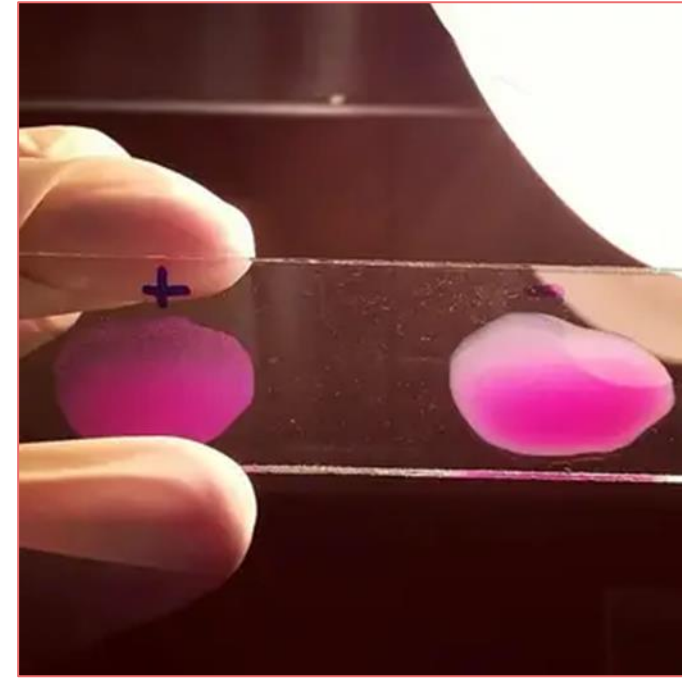
# Laboratory diagnosis of Brucellosis:

No.	Methods	Time Consuming
<u>Serology level</u>		
1	Rose Bengal test by Rapid Slide agglutination (screening) test	2 min
2	Rose Bengal test by Tube Agglutination test	2-4 hours
3	Brucella IgG/IgM by Immunochromatographic assay	5 minutes
4	2 Mercaptoethanol Test	15 minutes
5	ELISA (enzyme-linked immunosorbent assay) (IgG/ IgM)	45 minutes – 2 hours

# Rapid Slide Agglutination Test:

## Principle:

- Using *Brucella abortus* smooth cells as a bacterial suspension, stained with Rose Bengal ( pink color) in a buffered acidic medium (PH 3.6- 3.7).
- After mixing equal parts of Rose Bengal antigen & serum, the appearance of colored agglutinations is observed in case of brucellosis. The sensitivity limit is 25 IU/ml.
- The test highlights IgG and is positive later (2 to 3 weeks ) and the accuracy of the technique is very high (>95%).
- False-negative reactions occur especially in the early stages of acute infection.





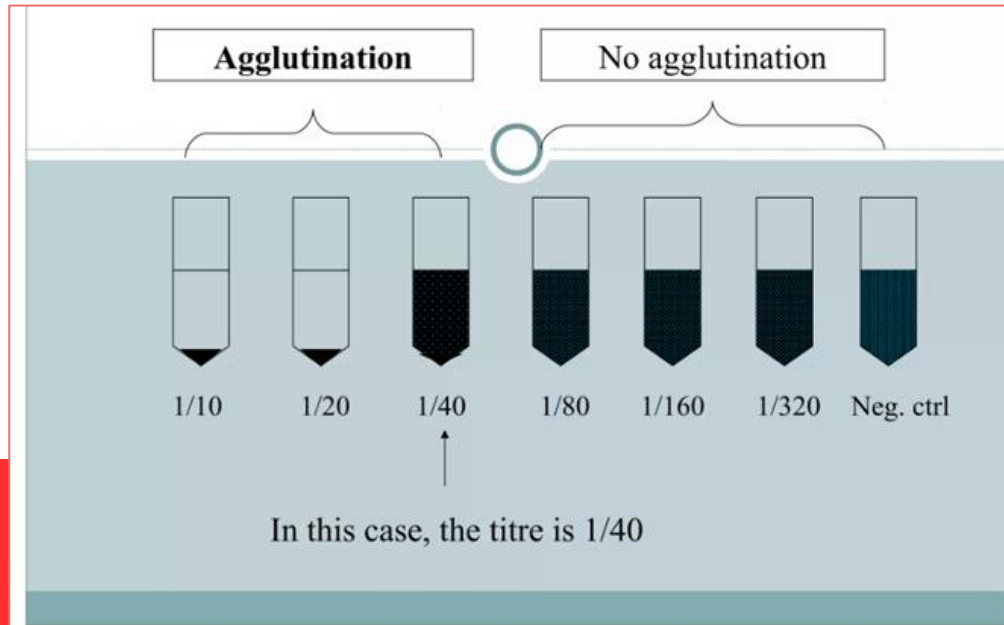
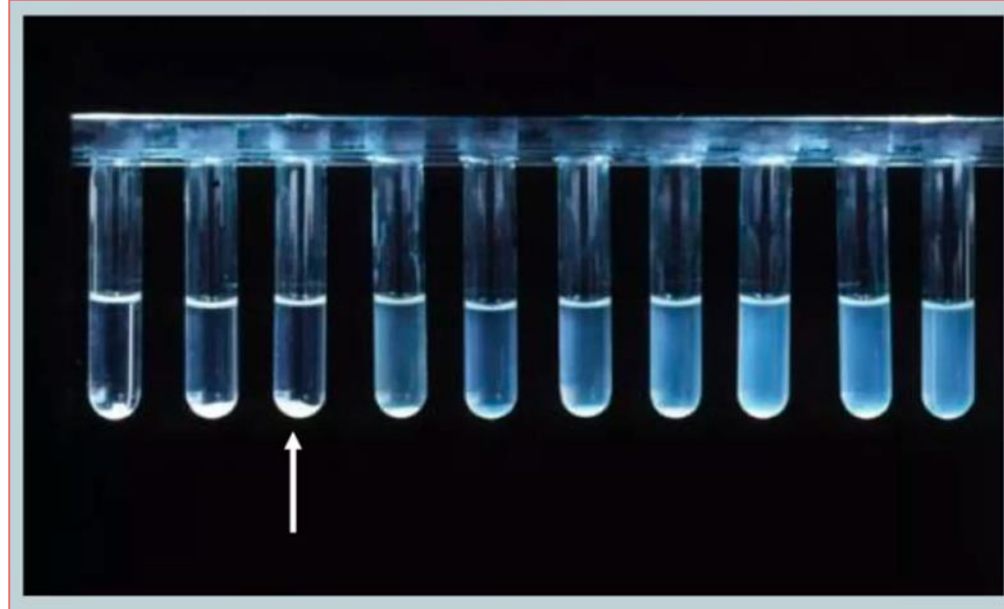
# Reading the result & Interpretation:

- \* No agglutination= absence of specific antibodies
- \*Agglutination (even slight) = presence of specific antibodies
- If agglutination appear after 15 seconds = (1:640)
- If agglutination appear after 30 seconds = (1:320)
- If agglutination appear after 1 min. = (1:160)
- If agglutination appear after 1.30 min. = (1:80)
- \*Patient history should be taken into account before giving the result.
- \*This test is a screening test only for the detection of Brucella agglutinins. If result is positive it must be confirmed by other serological tests for Brucellosis.

# Tube Agglutination Test (Serum Agglutination Test SAT)

## Principle:

- Identifies mainly the IgM antibodies.
- Equal volumes of serial dilutions of patient's serum & the antigen (killed suspension of standard strain of *Brucella abortus*) are mixed & incubated at  $37^{\circ}$  for 24hr.
- A single titer of more than 1:160 is evident of *Brucella* infection.



# Limitations of Rose Bengal Test:

There are a few reasons why diagnosing an *active* Brucella infection can be challenging:

- ✓ Some other types of bacteria can cause a false positive results.
- ✓ Immunizations may produce agglutinins capable of reacting with the antigens giving false positive results.
- ✓ A positive test doesn't always indicate a current infection. Previous exposure to Brucella might give false positive results.
- ✓ A very recent exposed to the Brucella antigen, might give false negative results due to low antibodies titer that cannot be detected.
- ✓ More tests or follow-up testing may be needed to confirm or rule out brucellosis.



# Widal Test

- Widal test is used for the diagnosis of enteric fever.
- Enteric fever refers to typhoid & paratyphoid caused by *Salmonella typhi* & *Salmonella paratyphi*.
- Typhoid fever is a significant cause of mortality across the world, with an estimated 12- 33 million cases occurring annually.
- Widal test is an inexpensive, easily accessible & non-invasive test to perform.



# Widal Test

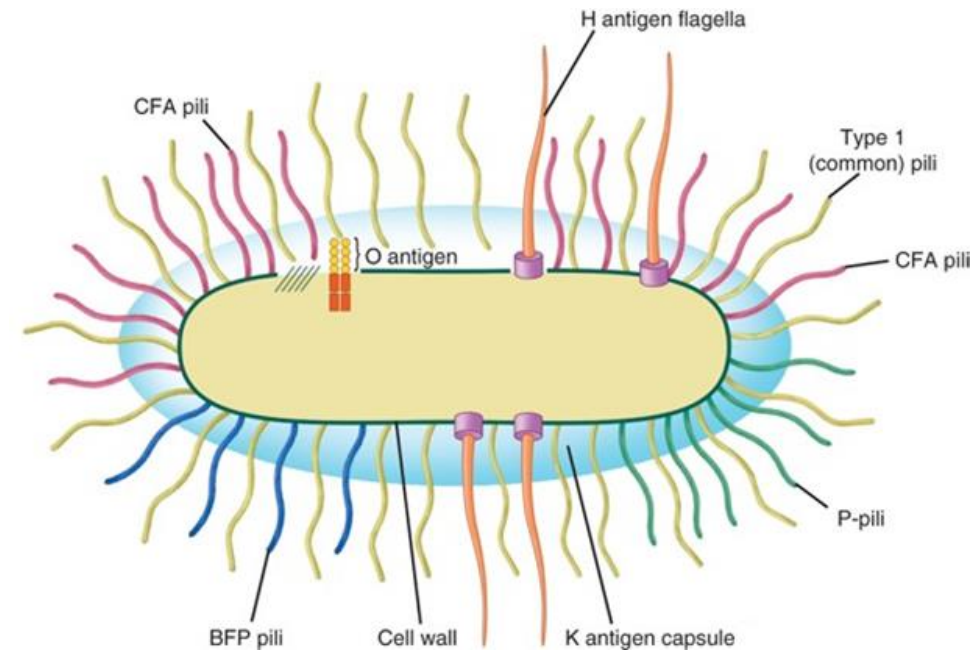
Salmonella Typhi antigens are:

## 1. O-Antigen (Somatic Antigen):

- These are 0-17 groups. 95% fall in group A, B, C, D, & E.
- O-Ag rises in 50% of the cases by the first week. It disappears in 6-12 months. This is diagnostic for acute infection.

## 2. H-Antigen (Flagellar Ag):

- H-Ag rises slowly and disappears after many years.



# Widal Test

## Principle:

- ❑ Widal test is an agglutination test for both qualitative & semi quantitative detection of anti- Salmonella antibodies in human serum.
- ❑ The patient's serum is allowed to react with suspension of known antigens. If specific antibodies are present agglutination will be observed.
- ❑ A serum sample with levels of agglutinating antibodies to O & H higher than 160 indicative of infection with that microorganism.
- ❑ This test can be done by:
  - Slide method
  - Tube method..(recommended).

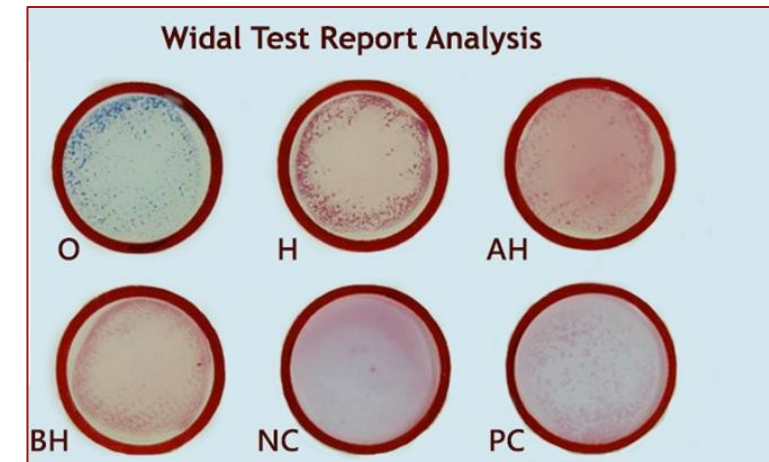


# Widal Test

## Slide Method:

### Procedure:

1. Bring the kit reagents & specimens to room temperature before use.
2. Gently shake the reagent to disperse the particles until you get a homogenous mixture.
3. Place 50  $\mu$ l of undiluted serum & 1 drop of positive & negative controls onto separated circles on the slide.
4. Add 1 drop (50  $\mu$ l) of the reagent next to serums drop.
5. Mix both drops spreading them over the full surface of the circle.
6. Place the slide on mechanical rotator (80- 100 rpm) for one minute.
7. Examine macroscopically for agglutination & compare the results with positive & negative controls.

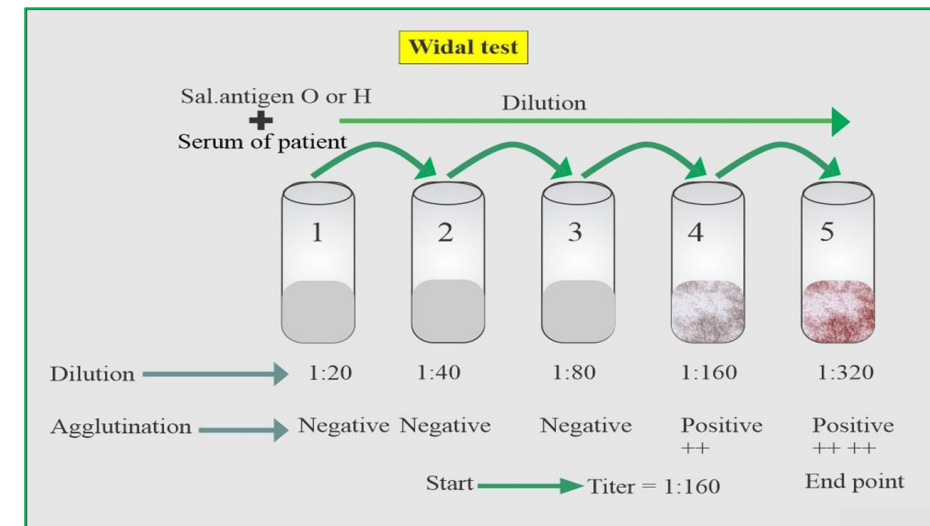
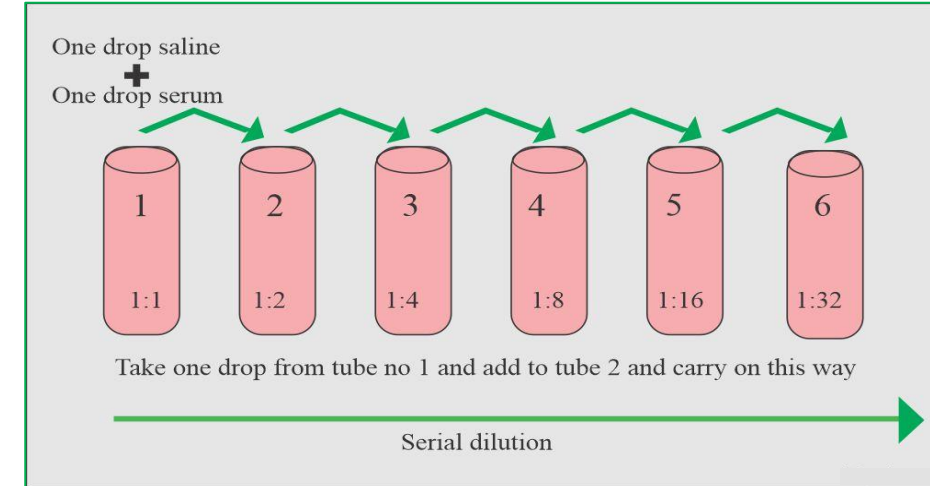


# Widal Test

## Tube Method:

### Results:

- ✓ The type of agglutination seen with O reactions is granular which is difficult to disperse, while the H reactions agglutination is slightly of uneven type usually described as floccular (fluffy).
- ✓ A diagnostic titer is the titer which is found during active phase of the disease.
- ✓ A titer of 1:80 (slide) or 1:160 (tube) or more is considered significant.





## Result Reporting:

- ✓ Widal test is reported by giving the titer for both O & H antibodies.
- ✓ The antibodies titer is taken as the highest dilution of the serum in which agglutination occurs.
- ✓ If no agglutination occurs, the test should be reported as:  
Typhi O titer < 1: 20  
Typhi H titer < 1: 20
- ✓ Significant titer is > 1:160 but may vary from one population to another.
- ✓ Titer of O antigens > 1:160 indicates recent infection.
- ✓ Titer of H antigens > 1:160 indicates past infection or vaccination.
- ✓ False +ve results may indicate immunization or a previous infection with Salmonella Typhi.



# Widal Test

## Interpretation of the Results:

- No value if done before 7 days of the onset of fever. Two or preferably more tests are done every 3-5 days to see a rising titer.
- 70% of adult patients will show a significant rise in the titer after 7-10 days of infection.
- It is very important to interpret the results after the recommended incubation time, otherwise false negative results may occur.
- Many people without the disease have agglutinin in low titer.
- The immunized person will also show agglutinin (antibodies). So a significant level is a four-fold rise in O-Ag or at least O Ag is  $> 1:80$  (slide method).
- Antibiotic use prevents the rise in antibody levels, thus false -ve results may occur.
- The slide test is suitable for screening, but confirmation should be done by tube method.

# Pregnancy Test

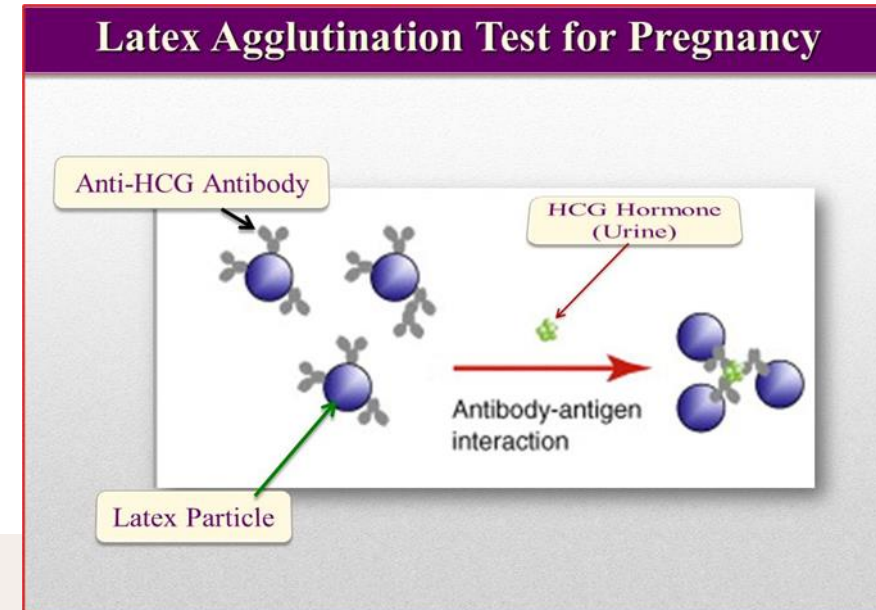
- ❑ Pregnancy tests are based on the detection of **human chorionic gonadotropin (hCG)**.
- ❑ hCG is a glycoprotein hormone secreted by the developing placenta shortly after fertilization.
- ❑ While hCG is a dependable marker of pregnancy, it cannot be identified till after implantation (6-12 days after conception).
- ❑ The pregnancy test is a rapid, highly sensitive, specific, qualitative & semiquantitative test.



# Direct Latex agglutination Method

## Principle:

- hCG has two units, alpha & beta. The alpha is antigenically similar to alpha subunits of LH, FSH & TSH. Consequently, the beta subunit is being used to yield a monoclonal antibody reagent with no cross reaction with LH, FSH & TSH.
- Latex particles coated with anti-hCG antibodies are allowed to react with female urine. If hCG is present, agglutination will be observed.
- Quantitative blood or serum beta test might be used to measure hCG levels as low as 1 mIU/ml, while urine test strips detect the threshold of 10 mIU/ml to 100 mIU/ml.
- Qualitative blood tests normally have a threshold of 25 mIU/ml, and so are less sensitive than some home pregnancy strips.



# Pregnancy Test

## Direct Latex Agglutination Method

### Procedure:

- ☐ Bring the reagents & the samples to room temperature.
- ☐ Place 0.05 ml of urine into one circle of the slide.
- ☐ Place similar amount of +ve & -ve controls in separated circles.
- ☐ Gently mix the latex reagent vial to ensure homogeneity & add 1 drop to each of the circles.
- ☐ Mix the reagents & samples equally & spread over the complete area of the circle.
- ☐ Place the slide on mechanical rotator (80-100 rpm) for 2 min.
- ☐ Examine for agglutination macroscopically.
- ☐ Compare the results with both +ve & -ve controls.

# Direct Latex Agglutination Method

## Results:

- ✓ A positive reaction will show agglutination reaction within 1-3 minutes.
- ✓ A concentration of hCG  $\geq 0.3$  IU/ml indicate positive results, while a concentration  $< 0.3$  IU/ml means negative results.

## Semi-quantitative test:

- ✓ Positive tests in qualitative method are to be proceeded to semi-quantitative test.
- ✓ A serial dilutions of serum samples are made.
- ✓ The highest dilution giving +ve results will be the titer.
- ✓ To calculate the concentration of hCG in 24hr. urine sample:  
multiply the volume of urine in 24hr. by the titer.
- ✓ Ex. : if the titer is 2.4 IU/ml & volume of 24hr. urine is 1500 ml, the hCG concentration will equal to 3500 IU/ml.

# Pregnancy Test

## Pregnancy Strip Test:

- ✓ A systemic review published in 1998 displayed that home pregnancy test kits, when used by skilled technicians are practically as accurate as professional laboratory testing (97.5-99%).
- ✓ When used by the customer, though, the accuracy fell to 75% due to fail to follow the instructions with the kit or misinterpreting the results.

## How to Use Pregnancy Tests

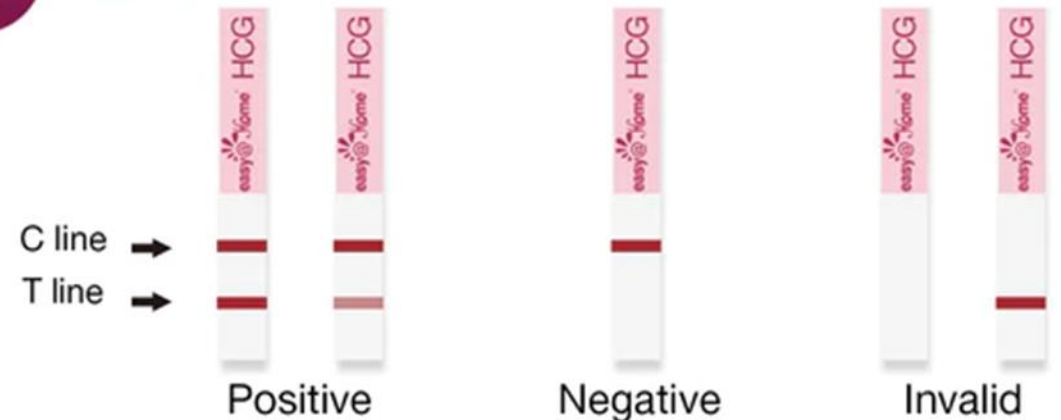
**01** **Dip** for 5-10 seconds until dye rises up



**02** **Wait** 3 minutes after lying test on white, flat, dry, non-absorbent surface



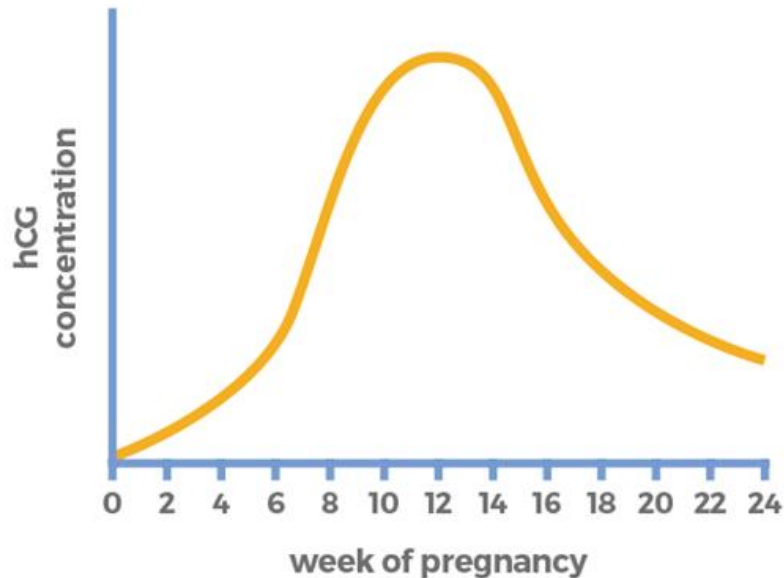
**03** **Read**





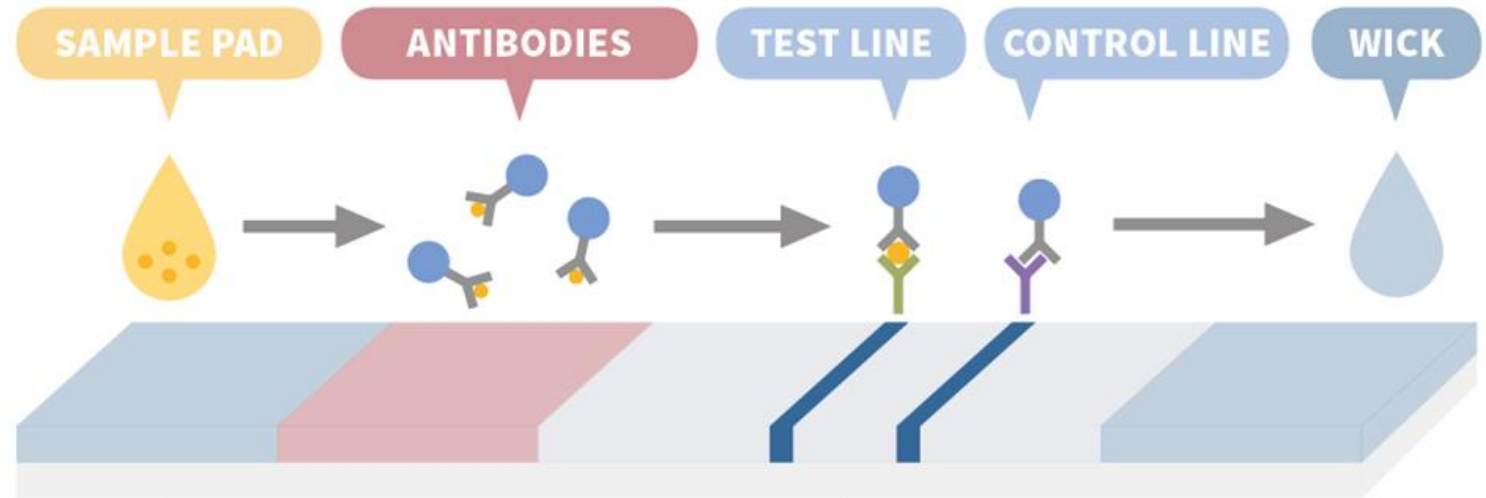
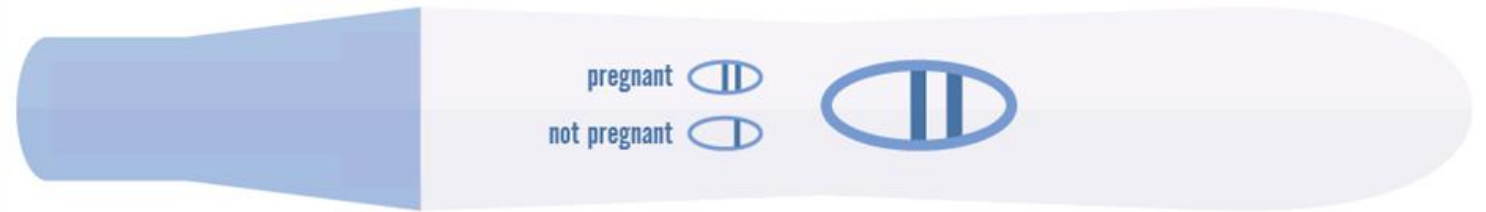
## WHAT DO PREGNANCY TESTS DETECT?

Pregnancy tests detect a hormone called human chorionic gonadotropin (hCG). This hormone is produced by the placenta from the time at which the embryo attaches to the uterus.



hCG is essential for the function of the corpus luteum, a temporary structure in the ovaries that produces the hormones progesterone and estrogen. It has also been linked to early pregnancy symptoms such as nausea and vomiting. hCG is eliminated in urine and can be detected by pregnancy tests around 9 days after fertilisation.

## HOW DO PREGNANCY TESTS WORK?



Urine applied to the sample pad. If a woman is pregnant, urine contains hCG.

hCG binds to mobile antibodies. These antibodies also have an enzyme attached to them.

Immobilised antibodies in the test zone bind to hCG. The enzyme on the first antibody changes the test line colour. Excess antibodies bind to immobilised antibodies in the control zone to show the test worked correctly.



## Pregnancy Test Accuracy:

- A **urine pregnancy test** is just as accurate as a blood test (99%), as long as you wait the recommended amount of time to take it. HCG doubles every couple of days in the very early stages of pregnancy.
- Urine tests can produce a false negative if the urine is **diluted** (from over-hydration) or the test is **taken too early**.
- As with home urine tests, it is possible (although rare) to end up with false results (both negative and positive) from a **blood pregnancy test**.
  - **False Negative Results:**
    1. This result can occur if the blood pregnancy test was performed too early, when there is not enough hCG in the blood to detect a pregnancy.
    2. a false negative blood test can occur if there is a condition called gestational trophoblastic disease.

# Pregnancy Test Accuracy:

## ■ False Positive Results:

This happens very rarely:

1. Taking a medication that contains hCG (such as when undergoing fertility treatments) or due to certain medical issues.
2. Possible causes of a false positive may include:
  - Antibodies present in the blood due to exposure to certain animal products (called heterophile antibodies)
  - Blood or plasma transfusion
  - Taking hCG for weight loss, doping, or fertility
  - IgA deficiency
  - Kidney failure
  - Rheumatoid factors
  - Some types of cancer



**Thank you  
for your  
Attention**



# **Serological Tests**

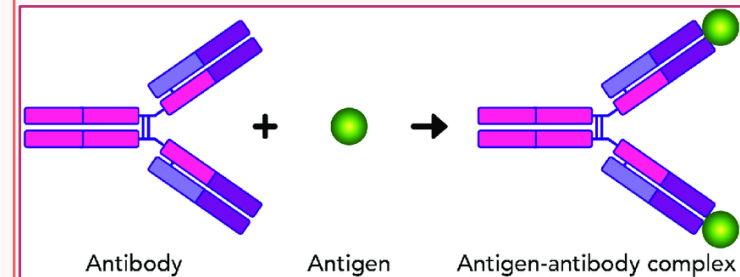
## **VDRL, ASO- Titer, CRP- Test**

## **Lec. 6**

**Dr. Ghaidaa S. Al- Bana**

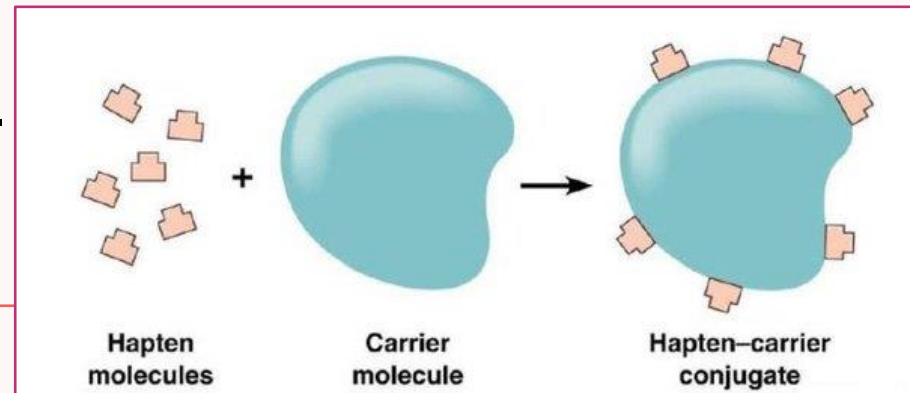
# Introduction

- Serological tests are blood tests that are performed to trace the antibodies in blood. Various serological tests are conducted for diagnosing different disease conditions. But these tests have one thing in common, **all these test focus on proteins produced in immune system.**
- Serological tests take the advantage of the specificity of antigen- antibody binding.
- Serological tests are frequently useful when:
  - The microorganism is difficult to isolate.
  - A previous infection needs to be documented.



# Definitions

- ❑ **Antigen:** An antigen is a substance that stimulates antibody formation & has the ability to bind to an antibody.
- ❑ **Hapten:** Is a low- molecular weight, non-antigenic substance that, when combined with an antigen, changes the antigenic specificity of that antigen.
- ❑ **Antibody:** is a glycoprotein substance (immunoglobulin) that is produced by B lymphocytes in response to an antigen.
- ❑ Antibodies may be monoclonal or polyclonal.

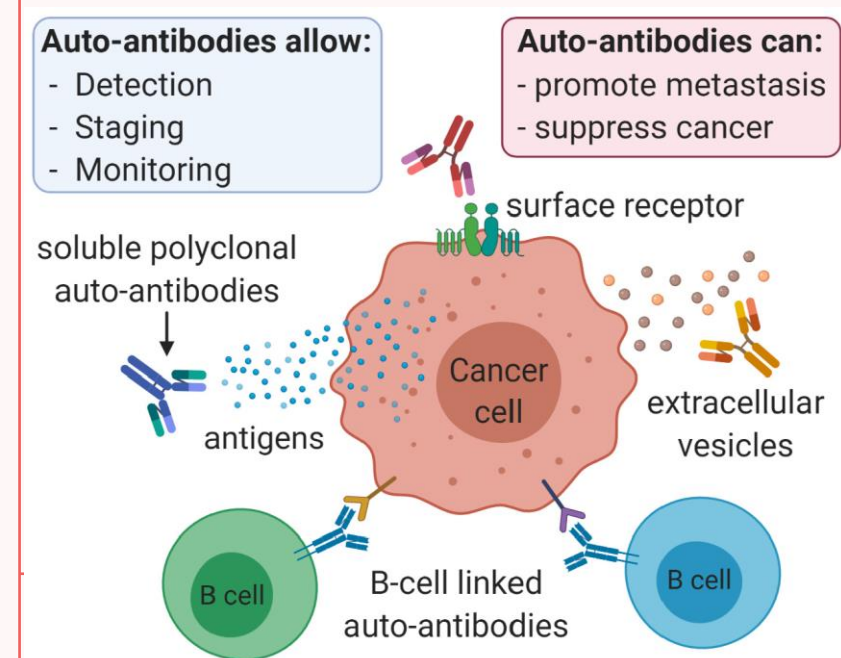


# Definitions

1. **Monoclonal antibodies:** are derived from a single B-cell clone and are produced as a single class of immunoglobulin with specificity unique to the antigenic stimulus.
2. **Polyclonal antibodies:** are produced as different classes of immunoglobulins by many B-cell clones in response to an antigen.
3. Antibodies produced in response to antigens from another species are called **heteroantibodies or xenoantibodies**.
4. **Alloantibodies:** are formed in response to foreign antigens from individuals of the same species.

## 5. Autoantibodies:

- ✓ are produced by the body's immune system against "self" antigens.
- ✓ Self-antigens against which autoantibodies are generated mainly include proteins, carbohydrates, fats, or nucleic acids.
- ✓ In clinical setups, serum autoantibody level has become a potent diagnostic biomarker for autoimmune diseases.
- ✓ Besides autoimmune diseases, autoantibodies can be detected in the serum sample of people suffering from cancer or having severe tissue damage.





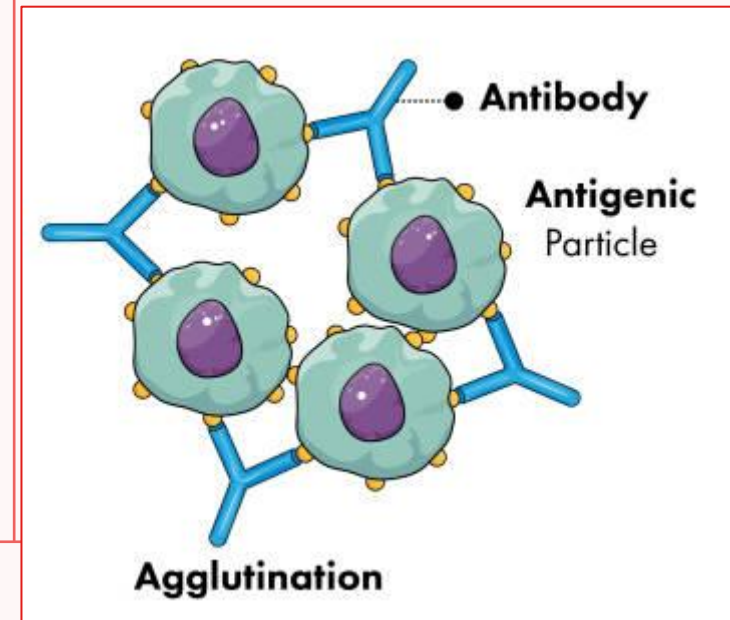
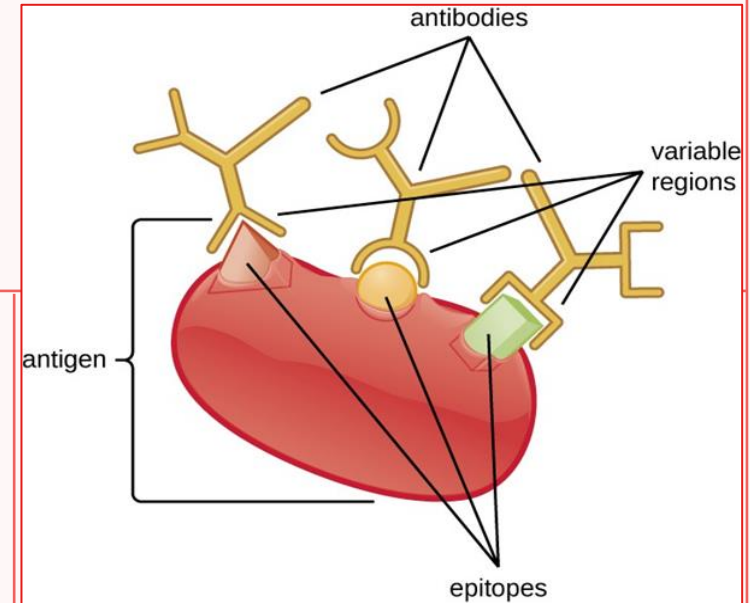
# Definitions

## Antigen-antibody reactions

I. **An epitope** is the part of an antigen that reacts specifically with an antibody or T-cell receptor.

A single antigen can have multiple epitopes for different, specific antibodies.

II. **Agglutination** is the clumping of particulate antigens by antibodies specific for the antigens.



# Definitions

## Antigen-antibody reactions

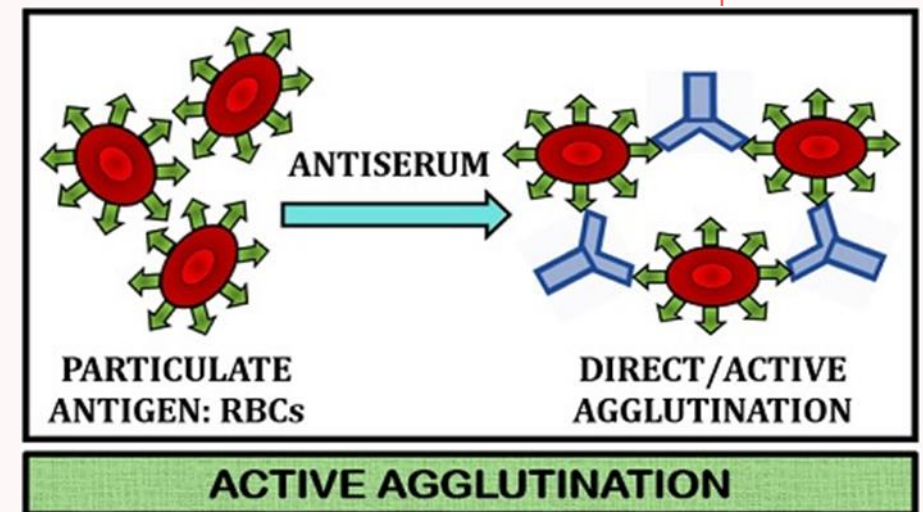
- III. **Affinity** is the tendency that an epitope has for combining with the antigen-binding site on an antibody molecule.
- IV. **Avidity** is the strength of the bond between the antigen and the antibody.
- V. **Sensitivity** is the smallest amount of antigen or antibody that can be detected.
- VI. **Specificity** is the ability of an antibody to bind to an antigen with complementary determinants and not to an antigen with dissimilar determinants.

# Techniques in Serology

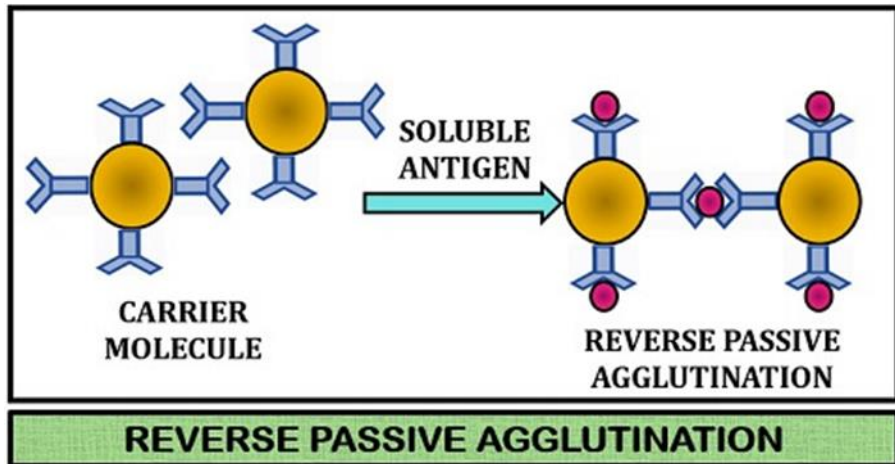
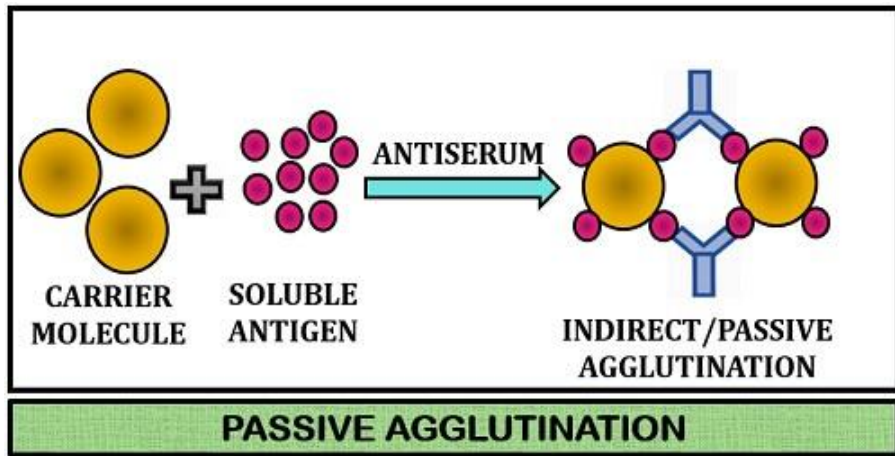
**A. Agglutination assays** demonstrate the presence of antigen-antibody reactions by the visible aggregation of antigen-antibody complexes. These tests are simple to perform and are often the most sensitive test method.

## 1. Active Agglutination:

It involves direct interaction of antibodies present in the serum with the particulate antigens carrying epitopes of interest. Example: Slide agglutination test (Blood-grouping), Widal test for the diagnosis of typhoid fever.



# Types of Agglutination Reaction



## 2. Passive Agglutination:

It involves indirect interaction of the antibodies with the soluble antigens via carrier particles. Passive agglutination primarily involves the binding of soluble antigen with the carrier matrices like latex bead, polystyrene etc. In this type, the antigens do not carry epitopes of interest, which have to be agglutinated.

## 3. Reverse passive agglutination:

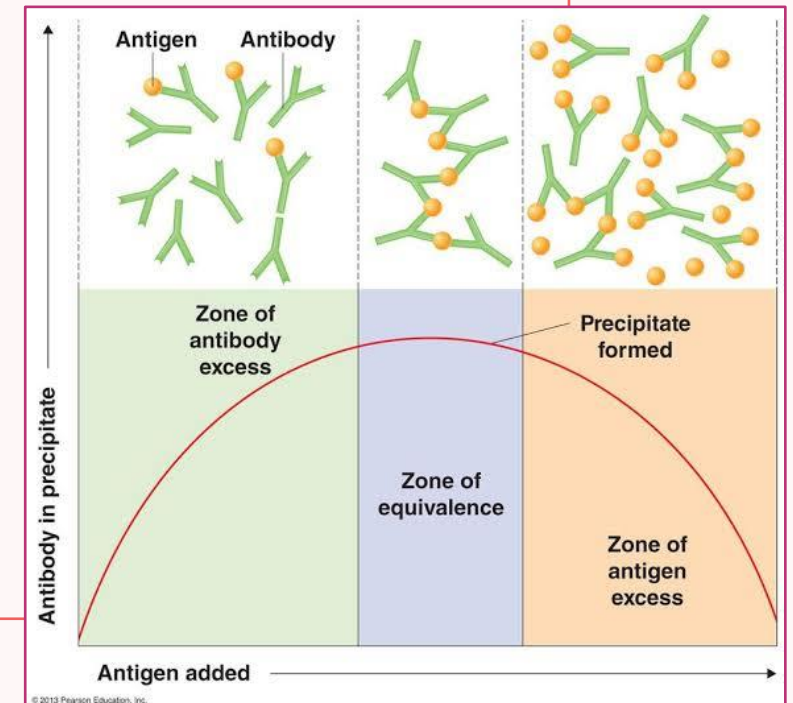
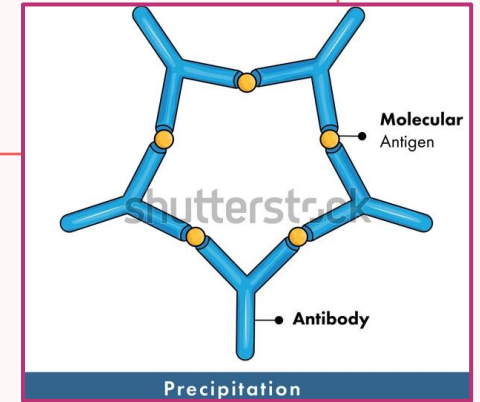
It uses known antibodies in place of antigen, which attach with the carrier matrix.

After the antibody's attachment with the carrier molecule, the antigens attach to the Fab sites of the antibody to agglutinate it. Example: Latex bead agglutination for both antigen and antibody.

# Techniques in Serology

## B. Precipitation assays:

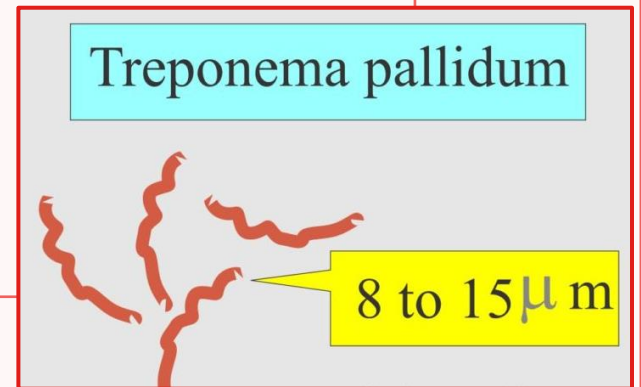
- When both are present in proper proportions, antigens and antibodies interact and form visible precipitates.
- The largest amount of precipitation is seen when antigens and antibodies are present in optimal proportions; this is known as the equivalence zone.
- False-negative reactions can occur when either antigen or antibody is present in excess.



# VDRL Test

## The Venereal Disease Research Laboratory (VDRL) slide test:

- ❑ It is a screening test for **syphilis** a sexually transmitted disease caused by the spirochete *Treponema pallidum*.
- ❑ It is a non- treponemal test (non- specific), which detects antibodies that act against cardiolipin as an antigen (ex. IgM & IgG antibodies to lipoidal material released from damaged host cells as well as to lipoprotein- like material, and to **cardiolipin** released from the treponemes).
- ❑ These antibodies are traditionally referred as **reagins**.

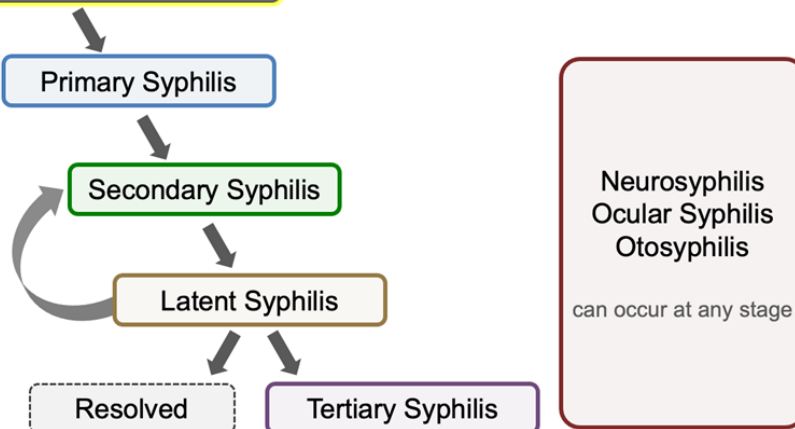




# Syphilis Serology

- ❑ Sexual contact with infected persons is the most common form of transmission of human syphilis. Transmission through blood or blood-product transfusion can occur but is rare now because of effective pretransfusion testing. In addition, syphilis can be passed from an infected pregnant woman to her fetus.

*Treponema pallidum* Infection



- ❑ There are four clinical stages of disease:

1. **Primary syphilis:** Inflammatory lesions (chancres) appear 2- 8 wks after infection. Serum tests for syphilis are positive in 90% of patients after 3 weeks.
2. **Secondary syphilis:** usually occurs 6- 8 wks after chancres first appear. This stage is characterized by a generalized rash, and secondary lesions may develop in the eyes, joints, or central nervous system (CNS). Serologic tests are positive in secondary syphilis.
3. **The latent stage:** of syphilis is contagious and is generally considered to begin after the second year of infection. There are no clinical symptoms, although serologic tests are still positive.
4. **Tertiary syphilis:** Approximately 80% of patients experience CNS involvement, which can result in paralysis or dementia.



# VDRL Test

## Principle:

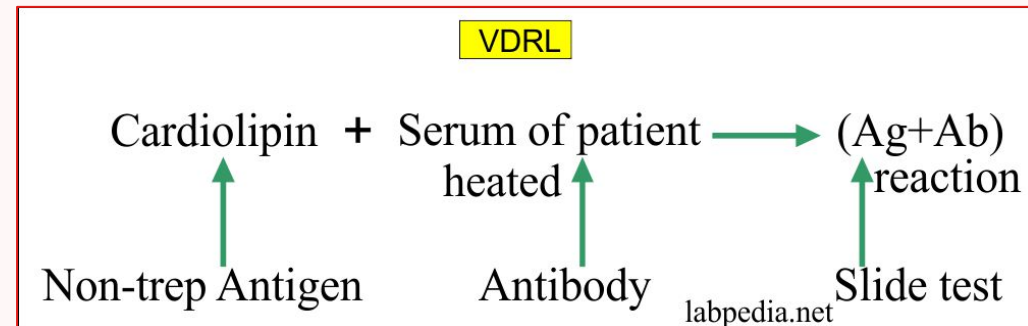
- ❑ VDRL test is a slide micro-flocculation test where patient antibodies react with cardiolipin antigen released by damaged host cells to form flocculates that can be detected using a microscope.

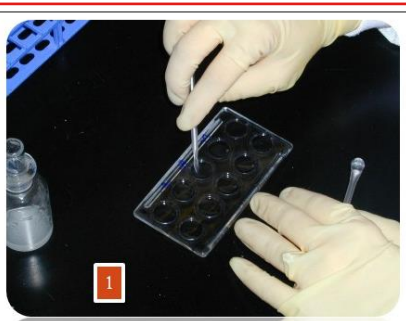
## Advantages:

- ❑ Not expensive.
- ❑ reproducible.

## Disadvantages:

- ❑ Not 100% specific for syphilis.
- ❑ Should be confirmed by other specific tests.
- ❑ Should run positive & negative control.





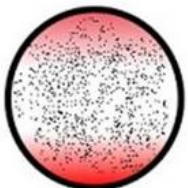
## Procedure:

❑ For the test, patient's serum is inactivated by heating at 56°C for 30 min in a water bath to remove non-specific inhibitors and complements.

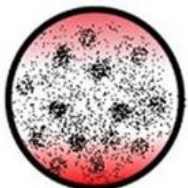
❑ A drop of antigen is placed on a slide, a drop of serum is then added to it. The slide is rotated to mix the content well.

❑ In a positive result, flocculation occurs within minutes.

❑ Clumping or agglutination indicates reactive specimen or the presence of autoantibodies' in patient's specimen, while non-reactive specimen will appear as homogeneous suspension.



Non-reactive



Weakly-reactive



Strongly-reactive

# VDRL Test

## Procedure:

➤ This test can be preformed both quantitatively & qualitatively. Those samples that are reactive by qualitative test are subjected to quantitative test to determine the antibody titers.

### ➤ **Qualitative test:**

1. 0.05 ml of inactivated serum is taken into one well.
2. 1 drop of cardiolipin antigen is then added to the well & rotated at 180 rpm for 4 min.
3. Every test must be accompanied with a known reactive & non- reactive controls.
4. The slide is then viewed under low power objective of a microscope for flocculation.
5. Depending on the size, the results are graded as nonreactive (NR), weakly reactive (WR), or reactive (R).

# VDRL Test

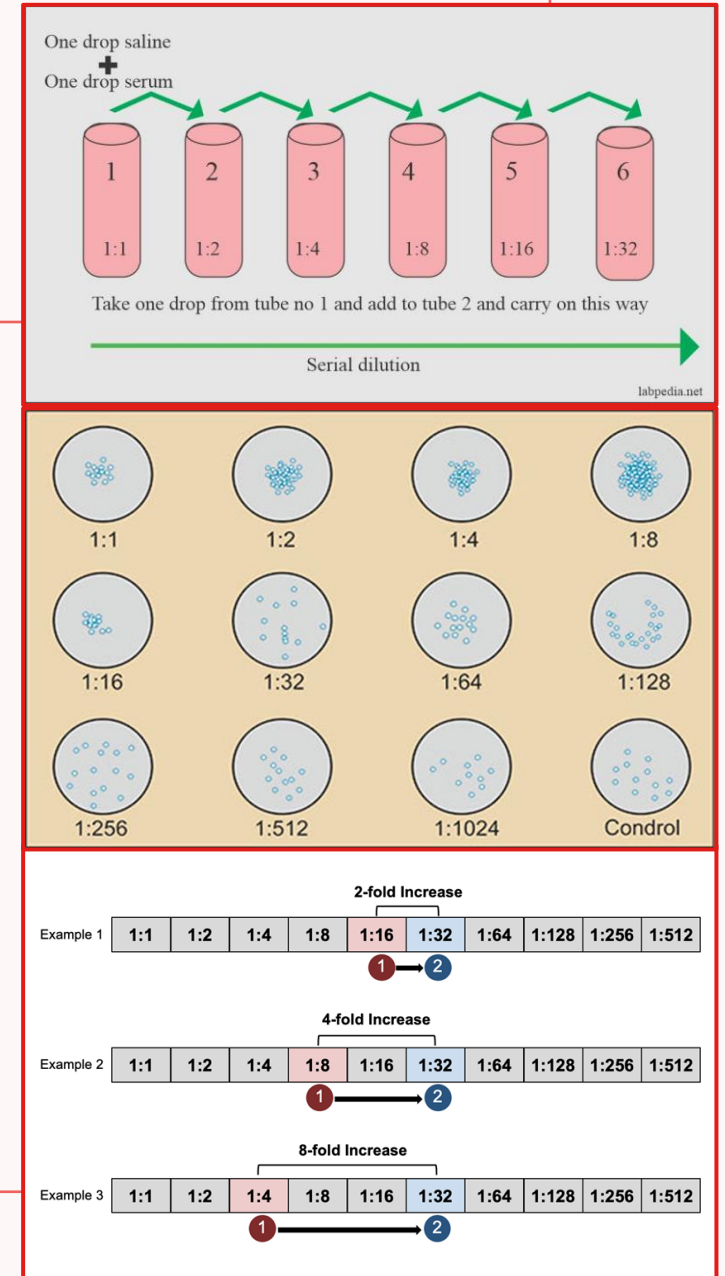
## ➤ Quantitative test:

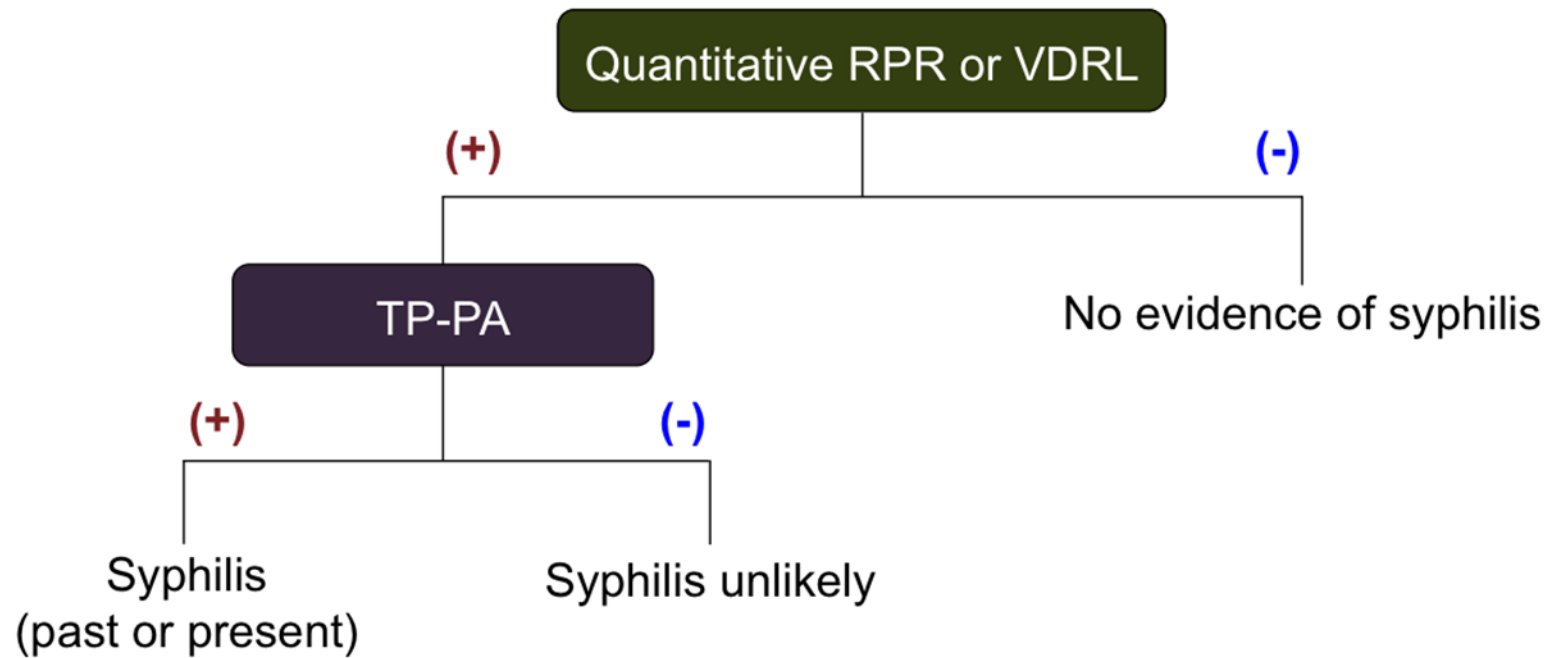
This is performed to determine the antibody titers.

1. The serum is doubly diluted in saline from 1:2 to 1:256 or more.
2. 0.05 ml of each dilution is taken in the well & 1 drop of antigen is added to each dilution & rotated.
3. The results is then checked under the microscope.
4. The highest dilution showing flocculation is considered as reactive titre.

➤ VDRL test may also be performed on CSF samples for the diagnosis of neurosyphilis.

➤ Quantitative VDRL is the test of choice for CSF specimens.



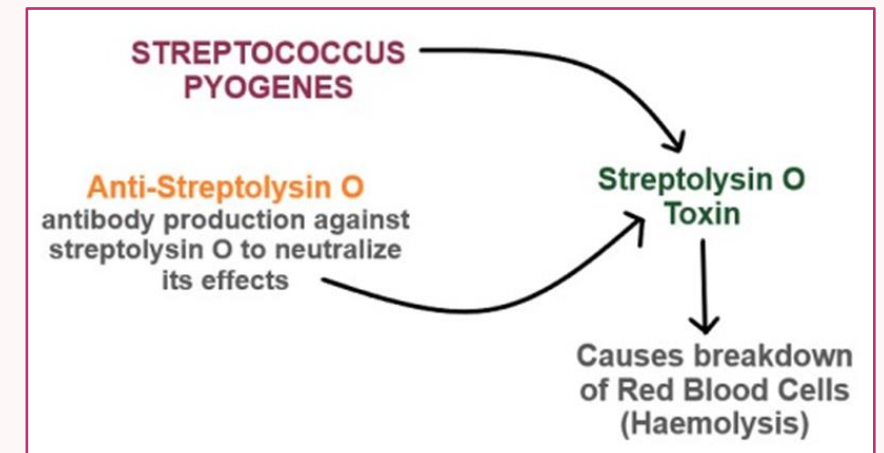


### Syphilis Serologic Screening

- The traditional (standard) serologic screening sequence algorithm uses a quantitative nontreponemal test (RPR or VDRL) for screening followed by a treponemal test for confirmation of positive screening tests.
- Abbreviations: RPR = rapid plasma reagin; VDRL = Venereal Disease Research Laboratory; TP-PA = Treponema pallidum particle agglutination.

# Streptococcal Serology

- ❑ Streptococcus pyogenes is a gram-positive coccus responsible for a number of human infections, some of which can have serious sequelae.
- ❑ Two hemolysins are produced by virtually all strains of *S. pyogenes*:
  1. **Streptolysin O (SLO)**: is an oxygen-labile enzyme that causes hemolysis by binding to cholesterol in the RBC membrane. It is antigenic, and the presence of antibodies to SLO is an indicator of recent streptococcal infection.
  2. **Streptolysin S**: is a nonantigenic, oxygen-stable enzyme. It causes hemolysis by disrupting the selective permeability of the RBC membrane.



## Laboratory diagnosis

### Anti-streptolysin O (ASO) titer:

- ❑ During an infection with *S. pyogenes*, the SLO produced by the bacteria stimulates an immune reaction, and specific antibodies are formed. These antibodies neutralize the hemolytic activity of the SLO; this neutralization provides the basis for the most commonly used test in the detection of streptococcal infections.
- ❑ Information about the extent & degree of the infection can be obtained by performing the latex agglutination test of the serum antibodies level.
- ❑ ASO test is a latex agglutination test for qualitative screening & semi-quantitative determination of anti-streptolysin O antibodies in serum.
- ❑ Specimen should be **serum collected in plain tubes**.

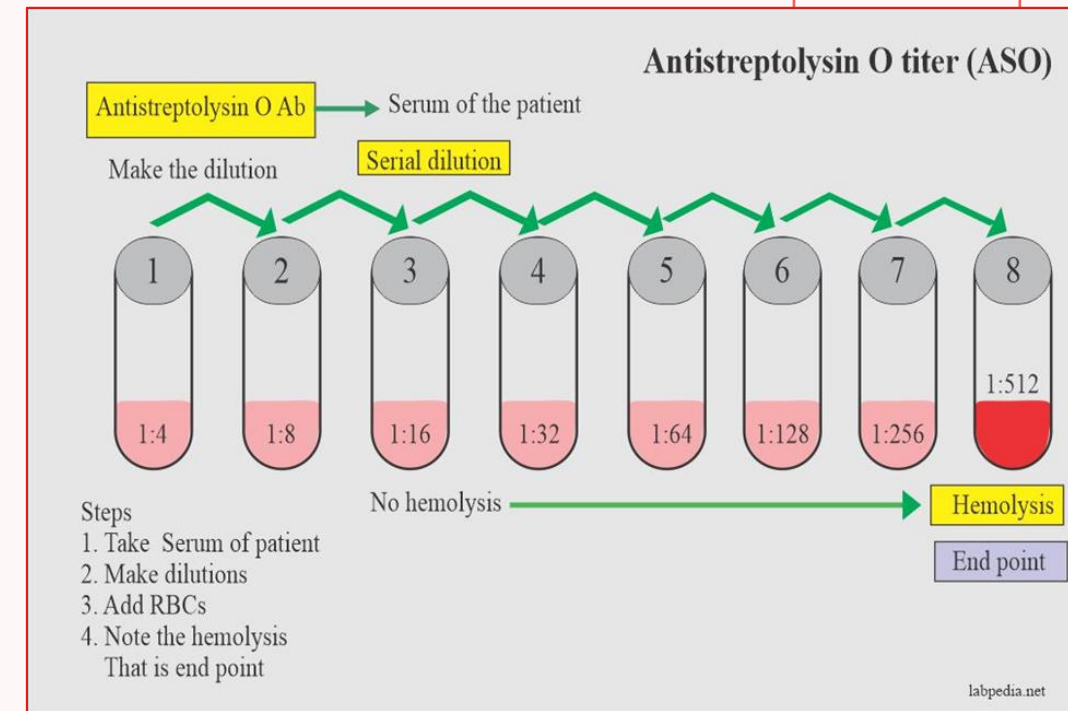


## Anti-streptolysin O (ASO) titer:

- ❑ The ASO titer begins to increase approximately 7 days after infection & peaks after 4 to 6 weeks.

### a. Principle:

- ❑ SLO is added to serial dilutions of patient serum, along with group O RBCs as indicator cells. If the patient serum contains antibodies against SLO, the antibodies will complex with the corresponding antigens within minutes. These complexes block the hemolytic activity of the antigen, and no hemolysis occurs. The ASO titer is reported as the reciprocal of the highest dilution that shows no hemolysis and is expressed in Todd units.



## b. Normal Values:

- ❑ ASO values vary widely among healthy individuals, making it difficult to establish normal values. Most healthy adults have ASO titers of less than 166 Todd units, with the usual titer decreasing after 50 years of age.
- ❑ The titer can remain elevated for weeks or months following acute disease.
- ❑ If the titer doesn't decrease with time, this indicates that a recurrent or chronic infection may exist.
- ❑ Performing additional tests should also be considered to confirm the results, like anti- DNase B test, which in combined with ASO titer, can detect up to 95% of streptococcal infections.

### ▷ Incidence of detect a positive result during the :-

○ 3rd week of infection	→	90-95 %	Incidence decreases as the time increases
○ 2 month	→	70-75 %	
○ 6 month	→	35 %	
○ 12 month	→	20 %	

### c. Results Interpretation:

- ❑ Agglutination appears within 3 min → Positive result (ASO level  $\geq 200$  IU/ml).
- ❑ No agglutination within 3 min → Negative result (ASO level  $< 200$  IU/ml).
- ❑ The approximate ASO concentration in serum sample can be calculated by utilizing the following formula:

$$[\text{ASO}](\text{IU/ml}) = \text{a reciprocal highest positive dilution} \times 200$$

(as the reagent sensitivity is 200 IU/ml).

- ❑ **e.g.** if the agglutination is present up to a titer of 1:8, then the approximate serum ASO level is:  $8 \times 200 = 1600$  IU/ml

#### ❑ Elevated ASO level may indicate:

- ▶ Active streptococcal infection
- ▶ Bacterial endocarditis
- ▶ Sore throat
- ▶ Rheumatic fever
- ▶ Scarlet fever
- ▶ Post- streptococcal glomerulonephritis

# Acute Phase Proteins Serology

- ❑ Acute phase proteins are a group of plasma proteins whose levels increase significantly and independently during the acute phase of an inflammatory process.
- ❑ They are primarily produced in the liver by the parenchymal cells.

## I. Alpha1-Antitrypsin:

Is a serine protease inhibitor that inhibits the action of specific proteases that cause lung damage and pulmonary inflammation. Heterozygous deficiencies result in increased risk of liver disease, glomerulonephritis, and connective tissue diseases.

## II. Alpha2- macroglobulin:

Is a protease inhibitor that plays a role in the coagulation, fibrinolytic, and complement components of hemostasis.

## III. Fibrinogen:

Plays an active role in wound healing after tissue injury. It is primarily responsible for an elevated erythrocyte sedimentation rate (ESR).

# Acute Phase Proteins Serology

## IV. C- reactive protein (CRP):

- ✓ Is normally present in trace amounts in serum, but may increase to 1000 times normal in many inflammatory processes. It is the first of the acute reactive proteins to appear following tissue injury or inflammation.
- ✓ CRP levels can be used to assess disease severity and monitor therapy.
- ✓ Elevated levels are seen in rheumatic diseases such as RA, bacterial and viral infections, burn injuries, malignancies, tuberculosis, and renal transplantation.
- ✓ Levels also rise rapidly following myocardial infarction.
- ✓ The latex agglutination test is the most commonly used method for CRP testing.

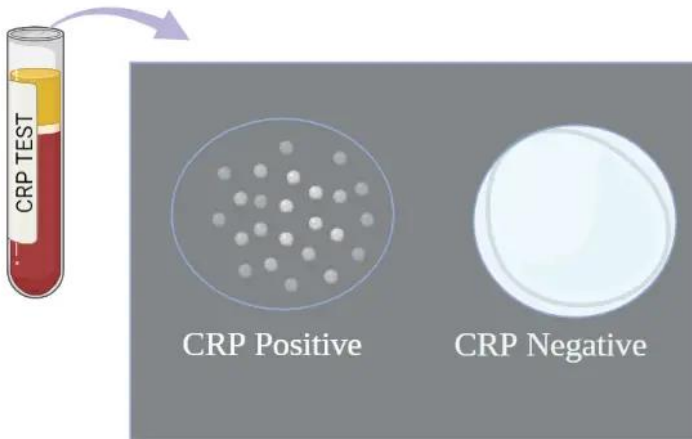
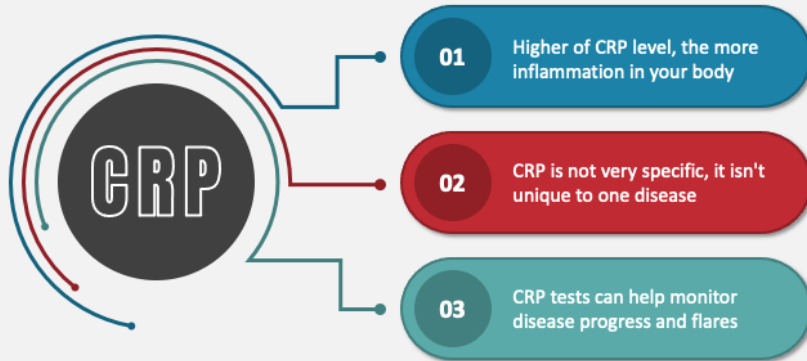
## V. Haptoglobin:

Is a plasma protein that binds to free hemoglobin. Decreased levels may be caused by intravascular hemolysis or decreased synthesis secondary to liver disease. Haptoglobin levels increase twofold to fourfold following tissue injury.

# CRP- Test

## C-REACTIVE PROTEIN (CRP)

Key Facts of C-Reactive Protein



**C-reactive protein (CRP) test**

## Principle:

- The CRP reagent is a suspension of polystyrene latex particles coated with the gamma globulin fraction of anti-human CRP specific serum.
- Agglutination is observed if CRP is present in a concentration of 6 mg/L or above, without previous sample dilution.
- The increased level of CRP indicates inflammation and tissue damage.
- The progression of an infection can also be monitored.

# C-REACTIVE PROTEIN (CRP)

## 1. Normal

0.5-3.0 Mg/L

## 2. Normal or Slightly Elevated

3-10 Mg/L

CRP levels naturally rise with age, & women tend to have slightly higher levels than men. Causes of slightly elevated CRP include mildly inflammatory conditions as well as chronic or long-term conditions such as

1. Diabetes
2. Periodontitis (Gum Disease)
3. Smoking
4. Obesity
5. Pregnancy

## 3. Moderately Elevated

10-100 Mg/L

Examples of Causes Include

1. Rheumatoid Arthritis
2. Cancer
3. Crohn's Disease

## 4. Markedly Elevated

>100 Mg/L

Examples of Causes Include

1. Major Trauma
2. Acute Bacterial Infection



# Thank you



# BLOOD UREA BLOOD CREATININE CREATININE CLEARANCE

## Lab. 3

Dr. Ghaidaa S. Al- Bana



# Introduction

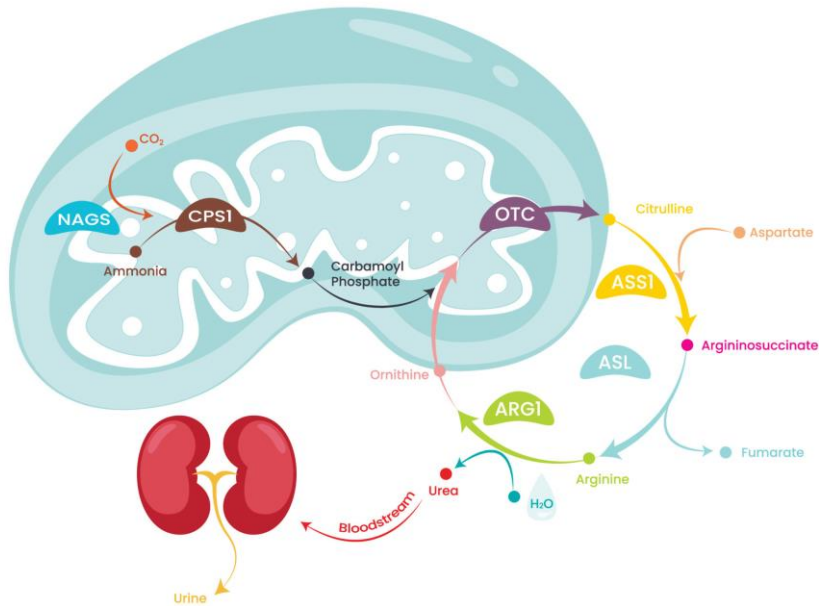
- The human kidney provides essential regulatory and excretory functions.
- Body water content, plasma electrolyte composition and plasma pH are all under the regulatory control of the kidney. In addition, the kidney provides a path of excretion for blood-borne, water-soluble, low-molecular-weight compounds. These include the end-products of protein metabolism, such as urea and creatinine.
- The renal blood flow is immense, constituting 25% of resting cardiac output.
- The glomeruli form 170–200 liters of ultrafiltrate per day and the selective reabsorption of water and solutes results in the final formation of approximately 1.5 liters of urine for excretion.

# Introduction

- Commonly used laboratory tests of renal function include:  
glomerular filtration rate (GFR), creatinine clearance, serum creatinine concentration, estimation of GFR, cystatin C assay, serum urea concentration, urinalysis, free water clearance and endocrine changes in renal disease.
- Urea is the principal nitrogenous waste product of metabolism and is generated from protein breakdown. It is eliminated from the body almost exclusively by the kidneys in urine, and measurement of its concentration, first in urine and later in blood, has had clinical application in the assessment of kidney (renal) function for well over 150 years.

# Urea Production & Excretion:

The Urea Cycle



- ❑ It is the principal nitrogenous end product of protein and amino acid catabolism. Proteins are first degraded to constituent amino acids, which are in turn degraded (deaminated), with production of ammonia ( $\text{NH}_3$ ), which is toxic.
- ❑ In a series of five enzymatically controlled reactions, known collectively as the “urea cycle”, toxic ammonia resulting from protein breakdown is converted to non-toxic urea.
- ❑ In addition to ammonia and the five “urea cycle” enzymes, endogenous production of urea requires the presence of bicarbonate, aspartate and energy input in the form of adenosine triphosphate (ATP).
- ❑ Carbon dioxide ( $\text{CO}_2$ ) is a secondary product of the urea cycle. Almost all of this urea production occurs in the cells of the liver (hepatocytes); the only other source is the cells of the kidneys.

# Blood Urea

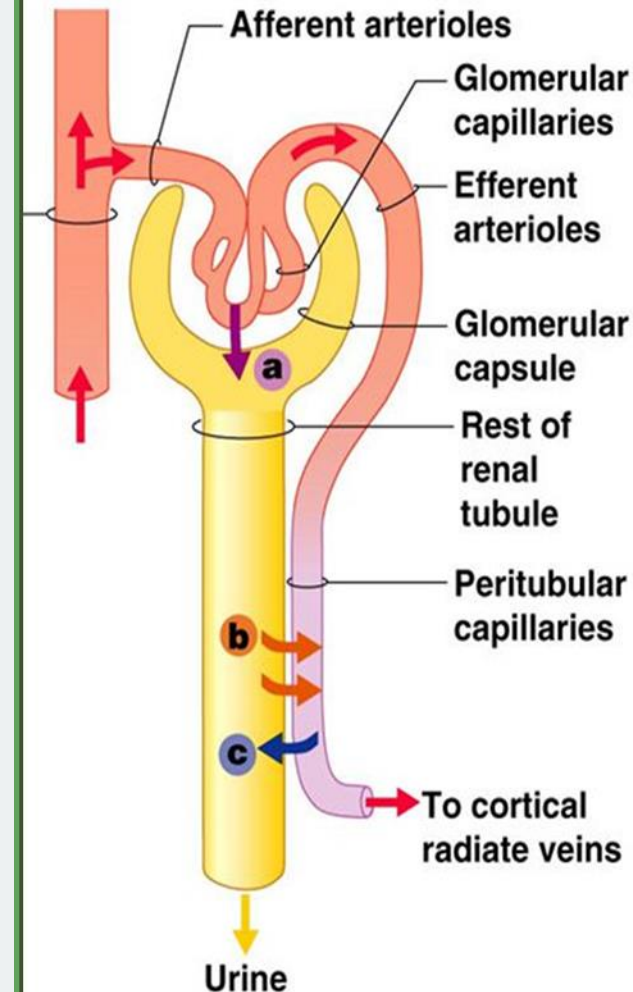
- The rate of urea production is influenced by protein content of diet; low-protein diet is associated with reduced urea production and high-protein diet is associated with increased urea production.
- Any pathology associated with tissue breakdown is for the same reason associated with increased urea production.
- A small amount (<10 %) of urea is eliminated via sweat and the gut, but most of the urea produced in the liver is transported in blood to the kidneys where it is eliminated from the body in urine.



# Blood Urea

- The process of renal elimination, begins with filtration of blood at the glomeruli of the approximately 1 million nephrons contained within each kidney.
- During glomerular filtration, urea passes from blood to the glomerular filtrate, the fluid that is the precursor of urine.
- The concentration of urea in the filtrate as it is formed is similar to that in plasma so the amount of urea entering the proximal tube of the nephron from the glomerulus is determined by the glomerular filtration rate (GFR).

## Urine Formation



### KEY:

- a** **Glomerular Filtration:** Water and solutes smaller than proteins are forced through the capillary walls and pores of the glomerular capsule into the renal tubule.
- b** **Tubular Reabsorption:** Water, glucose, amino acids, and needed ions are transported out of the filtrate into the tubule cells and then enter the capillary blood.
- c** **Tubular Secretion:**  $H^+$ ,  $K^+$ , creatinine, and drugs are removed from the peritubular blood and secreted by the tubule cells into the filtrate.

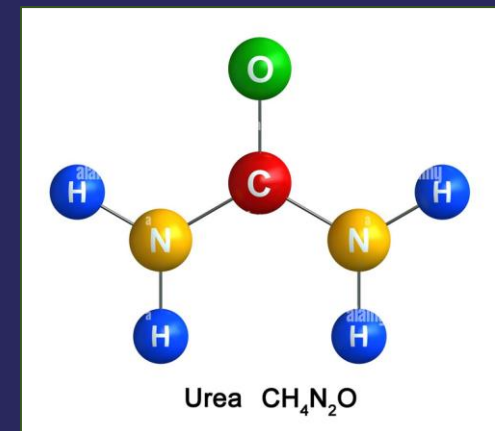


# Blood Urea

- Urea is both reabsorbed and secreted (recycled back into the filtrate) during passage of the filtrate through the rest of the tubule of the nephron; the net effect of these two processes results in around 30-50 % of the filtered urea appearing in urine.
- The facility of the kidney to adjust urea reabsorption and secretion as the filtrate passes through the tubule determines an important role for urea in the production of a maximally concentrated urine, when this becomes necessary.
- Although often considered simply a metabolic waste product, urea has two important physiological functions outlined above: detoxification of ammonia and water conservation.

# Measurement of plasma/serum urea

- Around the world, essentially the same method of urea analyses is used, but the result is expressed in two quite different ways. In the US and a few other countries, plasma or serum urea concentration is expressed as the amount of urea nitrogen.
- Although plasma or serum is used for the analysis, the test is still, commonly referred to as blood urea nitrogen (BUN), and the unit of BUN concentration is mg/dL.
- In all other parts of the world, urea is expressed as the whole molecule (not just the nitrogen part of the molecule) in SI units (mmol/L). Since BUN reflects only the nitrogen content of urea (MW 28) and urea measurement reflects the whole of the molecule (MW 60), urea is approximately twice ( $60/28 = 2.14$ ) that of BUN.
- Thus BUN 10 mg/dL is equivalent to urea 21.4 mg/dL.



## To convert BUN (mg/dL) to urea (mmol/L):

- multiply by 10 to convert from /dL to /L and
- divide by 28 to convert from mg BUN to mmol urea (i.e.  $10/28 = 0.357$ )

## So the conversion factor is 0.357

- BUN mg/dL multiplied by 0.357 = urea (mmol/L)
- Urea (mmol/L) divided by 0.357 = BUN (mg/dL)

## Approximate reference (normal) range:

Serum/plasma urea 2.5-7.8 mmol/L

Serum/plasma BUN 7.0-22 mg/dL



# Blood Urea

- It is widely accepted that there is an age-related increase in plasma/serum urea concentration but this is not well defined and there is uncertainty as to whether it simply reflects an age-related decline in renal function as some studies suggest, or occurs despite normal renal function as others seem to suggest.
- The results suggest that healthy elderly individuals (without any apparent loss of renal function), may have BUN levels as high as 40-50 mg/dL (14.3-17.8 mmol/L)].

# Causes of increased serum/plasma urea

- ❑ Serum/plasma urea concentration reflects the balance between urea production in the liver and urea elimination by the kidneys, in urine; so increased plasma/serum urea can be caused by increased urea production, decreased urea elimination, or a combination of the two.
- ❑ By far the highest levels occur in the context of reduced urinary elimination of urea due to advanced renal disease and associated marked reduction in glomerular filtration rate (GFR).
- ❑ GFR is a parameter of prime clinical significance because it defines kidney function. All those with reduced kidney function, whatever its cause have reduced GFR and there is good correlation between GFR and severity of kidney disease.
- ❑ The rate of decline in GFR distinguishes chronic kidney disease (CKD) and acute kidney injury (AKI). CKD is associated with irreversible slow decline in GFR over a period of many months, years or even decades; whereas AKI is associated with precipitous decline in GFR over a period of hours or days; AKI is potentially reversible.
- ❑ The value of urea as a test of renal function depends on the observation that serum/plasma urea concentration reflects GFR: as GFR declines, plasma/serum urea rises.

# The limitation of urea as a test of renal function

- In some circumstances plasma urea is not a sufficiently accurate reflection of GFR.
- For example, urea is an insensitive indicator of reduced GFR; GFR must be reduced by around 50 % before serum/plasma urea increases above the upper limit of the reference range.
- Furthermore, urea may be raised despite a normal GFR (i.e. normal renal function) so as a test of renal function, urea lacks specificity.

# Non-renal causes of increased plasma/serum urea

- ❑ The causes of increased plasma/serum urea in association with normal GFR include the *physiological* and the *pathological*. The two physiological causes are increased dietary protein and ageing.
- ❑ As previously mentioned, increase in dietary protein results in increased urea production. If sufficiently marked, this increased urea production can cause plasma/serum urea to rise. Mention has also been made of the age-related increase in plasma/ serum urea.
- ❑ Gastrointestinal hemorrhage is associated with increased protein intake (blood in the gut is effectively a high-protein meal) and thereby increased urea production and consequent increased plasma/serum urea.



# Non-renal causes of increased plasma/serum urea

- The role of urea in the maximal conservation of water by the kidney involves increased urea reabsorption and consequent tendency to mild increase in plasma/serum urea.
- Low circulatory states such as heart failure, dehydration, hypovolemic shock, necessarily invoke aspects of this adaptive response and all may be associated with mild increase in plasma/serum urea, despite normal GFR.
- Drugs that induce a catabolic state with increased protein breakdown and consequent increased urea production can cause plasma/serum urea to rise slightly; the most widely cited drug group to have this effect is the corticosteroids.

# Non-renal causes of increased plasma/serum urea

In general, the non-renal causes of increased plasma/serum urea result in a mild to moderate increase (usually no greater than 10.0 mmol/L (BUN 28 mg/dL). Concentration in patients with renal dysfunction can range from mildly increased to severely increased, depending on severity of disease.

Those with end-stage renal failure, requiring renal replacement therapy (dialysis, renal transplantation) may have plasma/serum urea >50.0 mmol/L (BUN >140 mg/dL).

The higher the concentration above 10 mmol/L, the greater is the chance that it is due to renal disease, but a slight increase cannot, of itself, be assumed to be due to a non-renal cause.

# Causes of reduced plasma/serum urea

- Reduced plasma/serum urea is less common and usually of less clinical significance than increased plasma/serum urea.
- Since urea concentration in plasma or serum reflects the balance between urea production and urea elimination in urine, reduced plasma/serum urea can be caused by decreased urea production, increased urinary urea excretion, or a combination of the two.
- There are two physiological causes of reduced concentration: low-protein diet, and pregnancy:

**Low-protein diet** is associated with reduced urea production and consequent tendency to reduced plasma/serum urea concentration.

The reduced plasma/serum urea that commonly occurs during **pregnancy** is due to the combined effect of reduced urea production and increased urea excretion. The increased urea excretion is consequent on increased GFR, a well documented physiological adaptation to pregnancy.

# Causes of reduced plasma/serum urea

➤ **Pathological cause** of reduced urea concentration is largely confined to **advanced liver disease**. This reflects the central role that the liver plays in urea production via the urea cycle.

**Inherited deficiency** of any one of the five enzymes of the urea cycle describes a rare group of conditions (called the urea cycle defects) that can give rise to reduced urea synthesis and consequent reduced plasma/serum urea concentration.

**Overhydration** induces increased GFR and consequent increased excretion of urea. For this reason overhydration, as might occur, for example, in the syndrome of inappropriate antidiuretic hormone (SIADH), is often associated with decreased plasma/serum urea.

# Serum Creatinine

- The best overall indicator of the kidney function is the **glomerular filtration rate (GFR)**.
- GFR is the rate in milliliters per minute at which substances in plasma are filtered through the glomerulus; in other words, the clearance of a substance from the blood.
- The normal GFR for an adult male is 90 to 120 mL per minute.
- The characteristics of an ideal marker of GFR are as follows:
  - I. It should appear endogenously in the plasma at a constant rate.
  - II. It should be freely filtered at the glomerulus.
  - III. It can be neither reabsorbed nor secreted by the renal tubule.
  - IV. It should not undergo extrarenal elimination.

# Serum Creatinine

- As no such endogenous marker currently exists, exogenous markers of GFR are used.
- Assessment of GFR using inulin, a polysaccharide, is considered the reference method for the estimation of GFR.
- It involves the infusion of inulin and then the measurement of blood levels after a specified period to determine the rate of clearance of inulin.
- Other exogenous markers used are radioisotopes such as chromium-51 ethylene-diamine-tetra-acetic acid ( $^{51}\text{Cr}$ -EDTA), and technetium-99-labeled diethylene-triamine-pentaacetate ( $^{99}\text{Tc}$ -DTPA).
- The inconvenience associated with the use of exogenous markers, specifically that the testing has to be performed in specialized centers, and the difficulty to assay these substances, has encouraged the use of endogenous markers.

# Serum Creatinine



- ❑ The most commonly used endogenous marker for the assessment of glomerular function is creatinine.
- ❑ Creatinine is the by-product of creatine phosphate in muscle, and it is produced at a constant rate by the body.
- ❑ For the most part, creatinine is cleared from the blood entirely by the kidney.
- ❑ Decreased clearance by the kidney results in increased blood creatinine.
- ❑ The amount of creatinine produced per day depends on muscle bulk. Thus, there is a difference in creatinine ranges between males and females with lower creatinine values in children and those with decreased muscle bulk.
- ❑ Diet also influences creatinine values. Creatinine can change as much as 30% after the ingestion of red meat.
- ❑ As GFR increases in pregnancy, lower creatinine values are found in pregnancy.



# Serum Creatinine

## ➤ Reference Values:

- Adult men: 0.9–1.3 mg/dL
  - Adult women: 0.6–1.1 mg/dL
  - BUN-to-creatinine ratio: 10:1 to 20:1
- Both plasma urea and plasma creatinine concentration are imperfect indices of GFR; neither analyte entirely fulfills the above criteria and both lack sensitivity to detect minimal change in GFR.
- Typically, GFR must be reduced by ~50 % before plasma urea or creatinine concentration rise above the upper limits of their respective reference range. By comparison with urea, however, creatinine more closely fulfills the above criteria and for this reason is the preferred test for assessment of kidney function.

## BUN & Creatinine

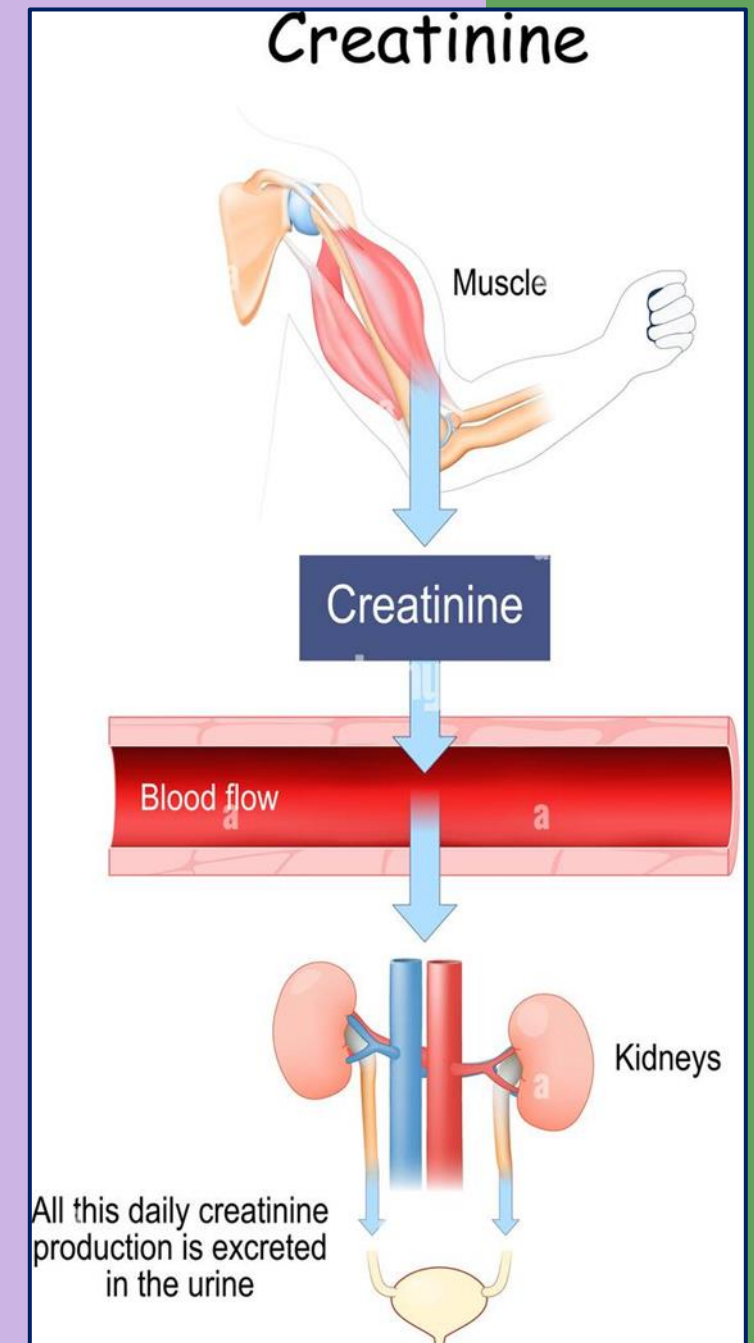
Characteristics	BUN	Creatinine
Definition	BUN is Blood urea nitrogen used by nephrologist to check the hydration status	Creatinine is a waste product of muscle and protein metabolism which is secreted at the glomerulus (a cluster of nerve endings around the end of a kidney tubule) and not absorbed back or again secreted by the renal tubule. Hence, it gives the correct measure of kidney function.
What does the test measure?	BUN test measures urea nitrogen in the blood	Creatinine test measures and assesses the kidney function
Affected by diet?	Compared to creatinine, BUN is affected by diet and is not suitable to be an indicator of kidney function.	Creatinine is not much affected by diet and is more eligible and suitable as an indicator of kidney function.
Production	In blood	In muscle and found in serum, plasma, and urine

# Creatinine Clearance

- ❖ The calculated clearance of creatinine is used to provide an indicator of GFR.
- ❖ This involves the collection of urine over a 24-hour period or preferably over an accurately timed period of 5 to 8 hours since 24-hour collections are notoriously unreliable.
- ❖ Creatinine clearance is then calculated using the equation:

$$C = (U \times V) / P$$

- ❖  $C$  = clearance,  $U$  = urinary concentration of a substance (mg/dl),  $V$  = urinary flow rate (volume/time i.e. ml/min),  $P$  = plasma concentration of the substance (mg/dl).
- ❖ Normal range:  
male = 85 – 125 ml/min, female = 75 – 115 ml/min



# Cystatin C

- ❑ Cystatin C is a low-molecular-weight protein that functions as a protease inhibitor produced by all nucleated cells in the body.
- ❑ It is formed at a constant rate and freely filtered by the kidneys.
- ❑ Serum levels of cystatin C are inversely correlated with the glomerular filtration rate (GFR). In other words, high values indicate low GFRs, while lower values indicate higher GFRs, similar to creatinine.
- ❑ The renal handling of cystatin C differs from creatinine. While glomeruli freely filter both, once cystatin C is filtered, it is reabsorbed and metabolized by proximal renal tubules, unlike creatinine. Thus, under normal conditions, cystatin C does not enter the final excreted urine to any significant degree. Cystatin C is measured in serum and urine.

# Cystatin C

- ❑ The advantages of cystatin C over creatinine are that it is not affected by age, muscle bulk, or diet, and various reports have indicated that it is a more reliable marker of GFR than creatinine, particularly in early renal impairment.
- ❑ Cystatin C has also been incorporated into eGFR equations, such as the combined creatinine-cystatin KDIGO CKD-EPI equation.
- ❑ Cystatin C concentration may be affected by the presence of cancer, thyroid disease, and smoking.

# Thank you

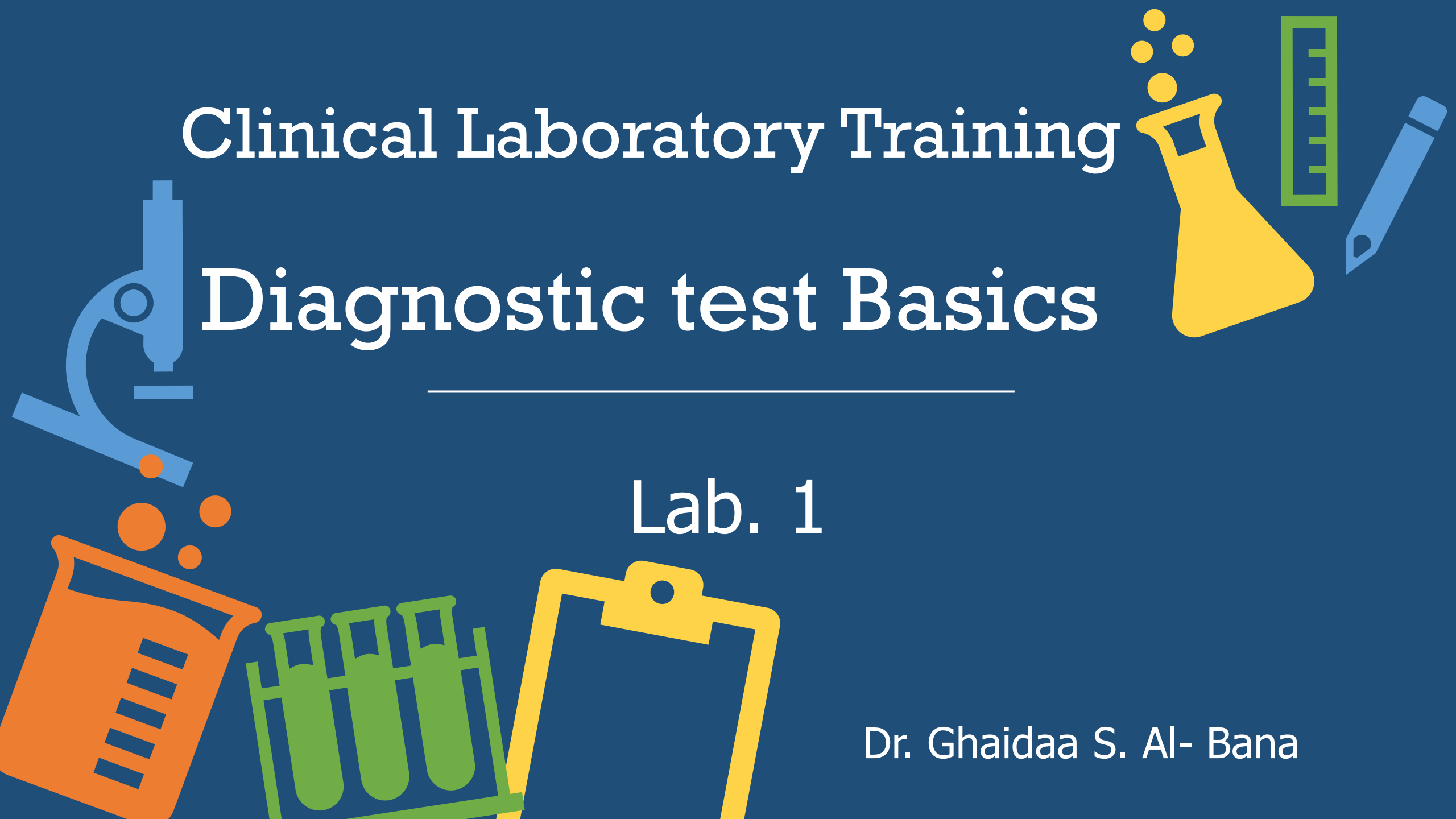
# Clinical Laboratory Training

## Diagnostic test Basics

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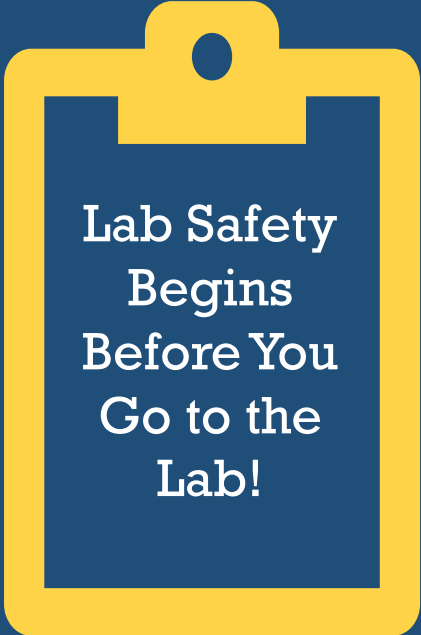
### Lab. 1

Dr. Ghaidaa S. Al- Bana



# Introduction:

- ❑ Laboratory tests contribute vital information about a patient's health. Correct diagnostic and therapeutic decisions rely, in part, on the accuracy of test results.
- ❑ Proper collection, identification, processing, storage, and transport of common sample types associated with requests for diagnostic testing are critical to the establishment of quality test results.



Lab Safety  
Begins  
Before You  
Go to the  
Lab!



# Preliminary Steps

There are four steps involved in obtaining a good quality specimen for testing: (1) preparation of the patient, (2) collection of the specimen, (3) processing the specimen, and (4) storing and/or transporting the specimen.

Prior to each collection, review the appropriate test description, including the specimen type indicated, the volume, the procedure, the collection materials, patient preparation, and storage and handling instructions.

**Preparing the Patient:** Provide the patient, in advance, with appropriate collection instructions and information on fasting, diet, and medication restrictions when indicated for the specific test.



# Preliminary Steps

Preparing the Specimen: All specimens submitted to the laboratory for testing must be properly labeled. Minimally, all specimens must be labeled with patient's full name and date and time of collection and origin (source) of specimen.

Specimen collection requires storage system that is capable of maintaining the optimal temperature for the diverse type of specimens. For example DNA storage at  $-20^{\circ}\text{C}$  and  $-80^{\circ}\text{C}$  can prevent DNA degradation for months or years.

◆ Mismatch of information between the label & the request,  
◆ inappropriate transport temperature, ◆ excessive delay in transportation, ◆ insufficient quantity (QNS)  $\longrightarrow$  Rejected Samples.



# Biological Specimens:

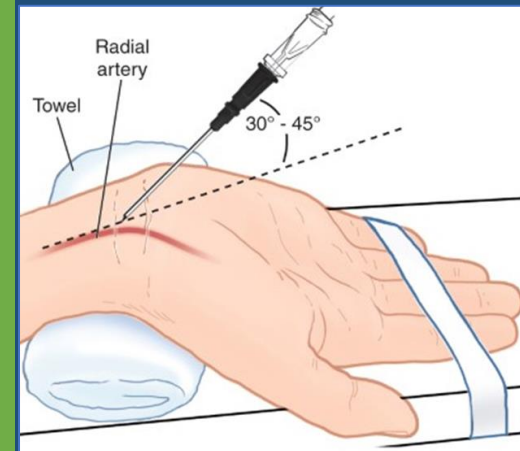
❑ Types of biological specimens that are analyzed in clinical laboratories include:

1. whole blood (venous blood, arterial blood, capillary blood).
2. serum
3. plasma
4. Urine
5. feces
6. saliva & sputum
7. spinal, synovial (joint), amniotic, pleural, pericardial, and ascitic (peritoneal) fluids.
8. various types of solid tissue (ex. stones, pancreatic pseudocysts).



# Blood Samples:

- ❑ Blood for analysis may be obtained from veins, arteries, or capillaries.
- ❑ **Arterial puncture** is used mainly for blood gas analyses (ABGs).
- ❑ Arterial blood:
  - Best specimen for evaluating respiratory function
  - Has high oxygen content & consistency of composition.
- ❑ ABGs:
  - I. Used in diagnosis & management of respiratory & metabolic disorders.
  - II. Provide valuable information about patient's:
    - ✓ Oxygenation
    - ✓ Ventilation
    - ✓ Acid-base balance



## Commonly Measured ABG Analytes:

- ❑ PH (7.35- 7.45): a measure of acidity or alkalinity of blood (acidosis or alkalosis).
- ❑ PaO<sub>2</sub> (80- 100 mm Hg): partial pressure of O<sub>2</sub> dissolved in arterial blood.
- ❑ PaCO<sub>2</sub> (35- 45 mm Hg): partial pressure of CO<sub>2</sub> dissolved in arterial blood.
- ❑ HCO<sub>2</sub> (22- 26 mEq/L): a measure of bicarbonate in the blood.
- ❑ O<sub>2</sub> sat. (97%- 100%): percent O<sub>2</sub> bound to hemoglobin.
- ❑ Base excess (-2- +2 mEq/L): a calculation of non-respiratory part of acid- base balance.

## Disadvantages of Arterial Puncture:

- Technically difficult.
- Potentially more painful & hazardous than venipuncture.
- Thus, not normally used for routine blood tests.

# Site Selection Criteria for Arterial Puncture:

## ☐ Presence of collateral circulation:

- Blood supply from more than one artery.

## ☐ Artery accessibility & size.

## ☐ Type of tissue surrounding puncture site:

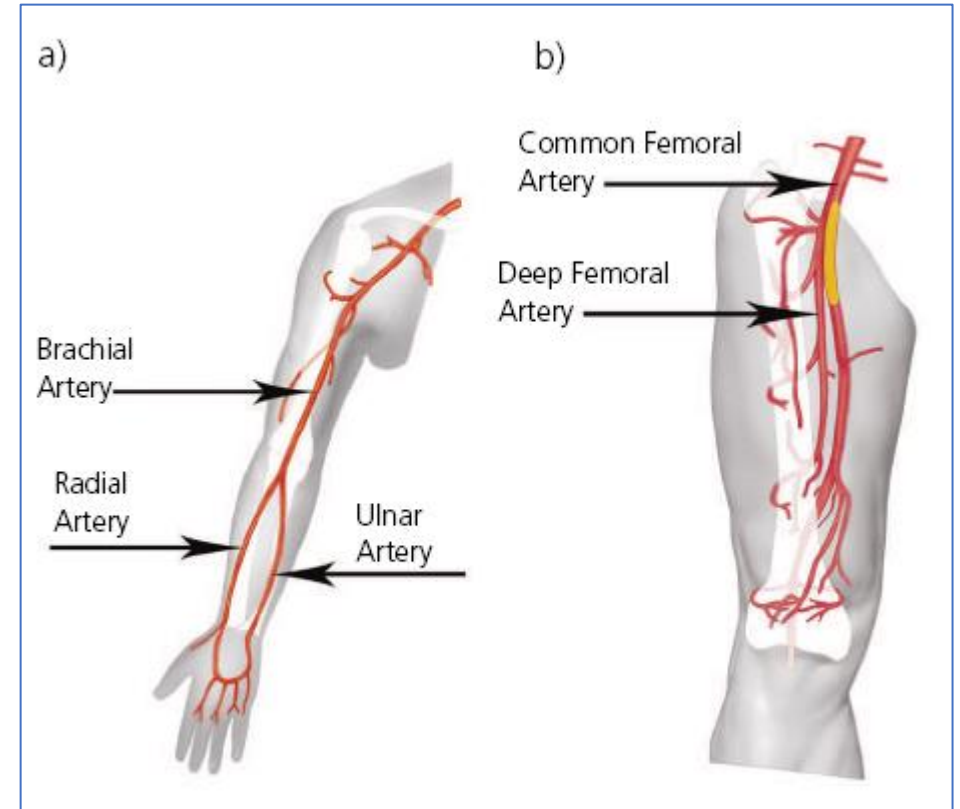
- Low risk of injuring adjacent structures or tissue during puncture.
- Ability to fix or secure artery to prevent rolling.
- Adequate pressure can be applied to artery after collection.
- Absence of inflammation, irritation, edema, hematoma, lesion, wound, or recent arterial puncture.

# Arterial Puncture Sites:

- ❑ Radial artery (most common).
- ❑ Brachial artery.
- ❑ Femoral artery.

• Radial artery is the most preferable site used because:

- I. It is easy to access.
- II. It is not a deep artery which facilitates palpation, stabilization & puncturing.
- III. It has a collateral blood circulation (radial & ulnar arteries).
- IV. Less chance of hematoma formation after collection.



## Main arteries:

(a) arm – radial and brachial arteries are puncture sites whereas collateral circulation to the hand is provided by the ulnar artery.

(b) groin – femoral artery is the puncture site (figure shows anatomically subdivided parts of the femoral artery – common and deep femoral artery).



# Blood Samples:

- ❑ Venous blood is usually the specimen of choice, and venipuncture is the method for obtaining this specimen.
- ❑ **Venipuncture** is defined as all of the steps involved in obtaining an appropriate and identified blood specimen from a patient's vein.
- ❑ The process of collecting blood is known as **phlebotomy** (from phleb, which means vein, and tome, to cut or incise) and should always be performed by a trained phlebotomist.



Equipment for venipuncture using a blue (23G) needle

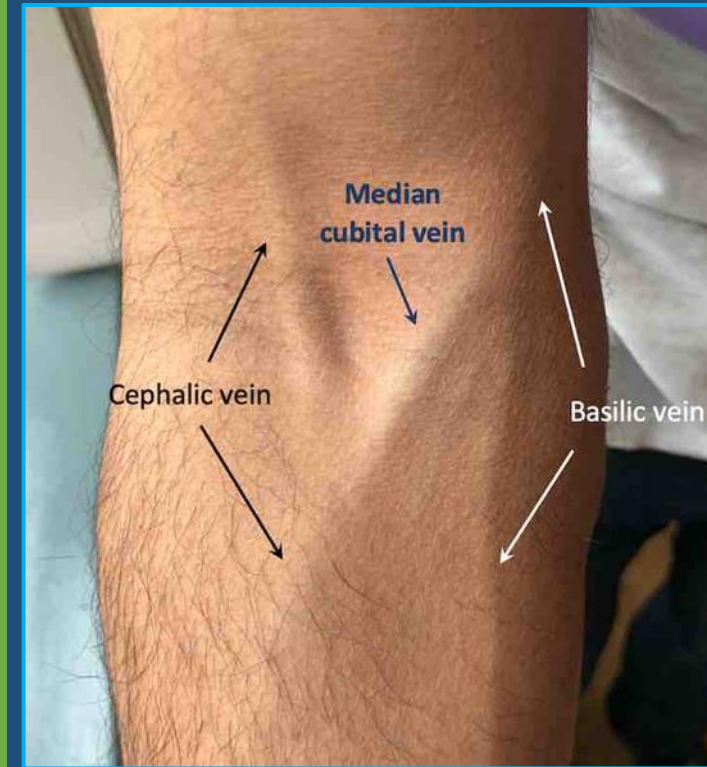
# Blood Samples:

- Venipuncture is one of the most routinely performed invasive procedures and is carried out for any of five reasons:
  1. to obtain blood for diagnostic purposes.
  2. to monitor levels of blood components.
  3. to administer therapeutic treatments.
  4. to remove blood due to excess levels of iron or erythrocytes (red blood cells).
  5. to collect blood for later uses, mainly transfusion either in the donor or in another person.



# Venipuncture Sites:








- ❖ Venipuncture sites are commonly found in the superficial veins of the upper limb:
- Median Cubital Vein: It lies in the anterior region of the elbow, within the cubital fossa, it is close to the surface of the skin and is an ideal option as it does not lie in close proximity to any large nerves.
- Cephalic Vein: found in the dorsal venous network of the hand. It ascends from the antero-lateral side of the upper limb, passing anteriorly at the elbow.
- Basilic and median antebrachial vein may also be used for the procedure.



Medial cubital vein in the right cubital fossa

# Common blood collection tubes

- ❖ **Light blue:** Used for hematological tests to assess clotting, including coagulation studies (e.g. activated partial thromboplastin time (aPTT), prothrombin time (PT) and international normalized ratio (INR), D-dimer and fibrinogen). Contains sodium citrate, a reversible anticoagulant.
- ❖ **Lavender:** Used for a variety of routine hematological tests, including full blood count, erythrocyte sedimentation rate (ESR), blood film and HbA1c. Contains EDTA, an anticoagulant.
- ❖ **Pink:** Used for group and save (if there is a possibility a patient may require a transfusion) and for cross-matching (to confirm blood donor compatibility prior to transfusion). Contains EDTA, an anticoagulant.

VACUTAINER TUBES		
Color	Anticoagulant	Uses
	No anticoagulant	Serological examination in biochemistry
	Sodium Fluoride	Glucose estimation
	EDTA @VijayPatho	Hematological examination like complete hemogram, ESR
	3.2% sodium citrate	Coagulation studies like PT, APTT
	Heparin	Bone marrow studies
	Citrate	Blood culture
	(K2)EDTA	Blood Bank tests. Blood typing. ABO grouping etc

- ❖ **Gold:** Used for a wide range of tests - for example in biochemistry (including urea and electrolytes, C-reactive protein, liver function tests, cardiac enzymes, lipid profile, amylase, bone profile), endocrinology (including thyroid function tests, beta-hCG), toxicology (ethanol and drug levels) and tumor markers. Contains silica, a clot activator, and a serum separator gel to separate the plasma and blood cells.
- ❖ **Grey:** Used for measuring glucose and lactate levels. Contains sodium fluoride (prevents glucose breakdown after taking the sample) and potassium oxalate, an anticoagulant.
- ❖ Other less commonly used blood collection tubes include: **red** (used rarely for biochemistry tests that cannot be stored in the **yellow** bottle, including certain hormone, toxicology and bacterial/viral serology), **green** (used to measure renin and aldosterone, neutrophil function tests and cytogenetics) and dark blue (used to measure trace metals such as zinc and aluminum).

# Draw Order:

- ◆ When multiple tubes are drawn, it is important to prioritize drawing order to help prevent contamination.
- ◆ The accepted draw order is:
  1. sterile blood culture tubes
  2. Light blue top tubes
  3. clot tubes (e.g, red-top tube, gold-top tube)
  4. anticoagulated tubes (e.g, green-top tube)
  5. lavender-top tube
  6. pink-top blood bank tube
  7. dark blue-top
  8. grey-top tubes are drawn last.





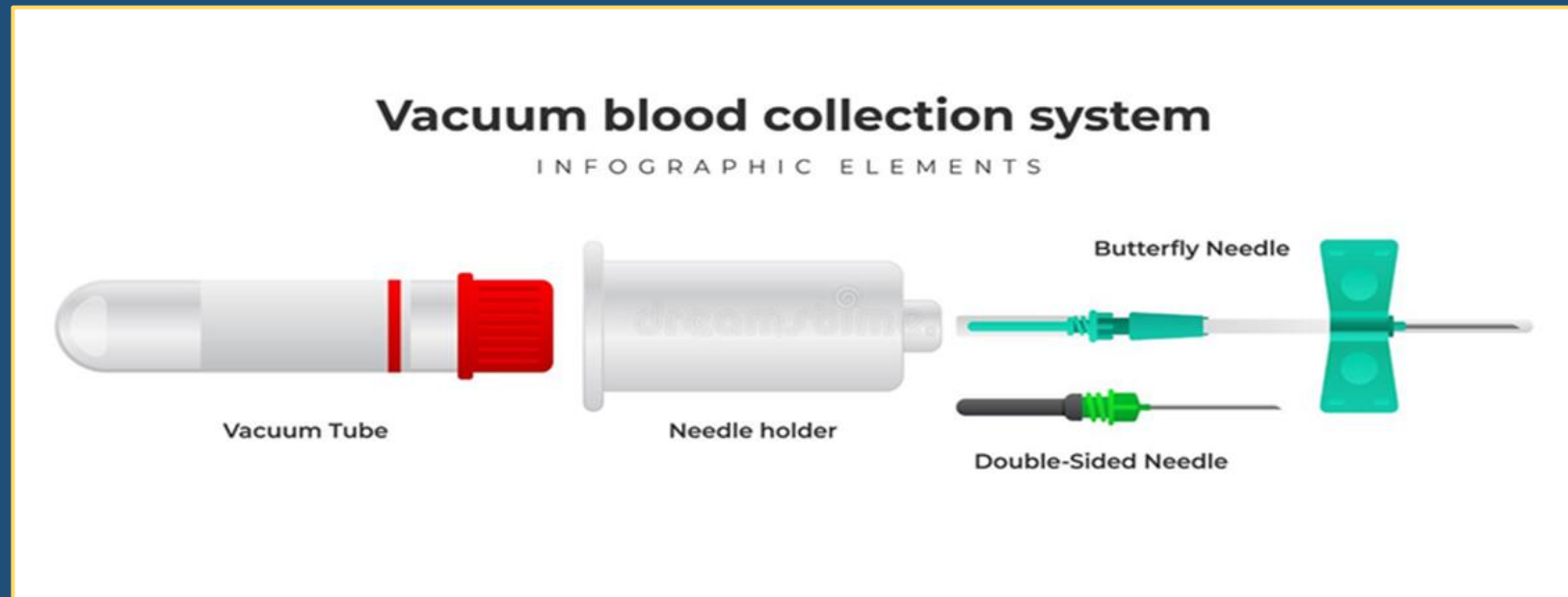
# Tube Inversion:

◆ Following blood collection, tubes need to be inverted to mix the blood with the additive. This should be done gently to prevent hemolysis of the sample:

- **Light blue:** Invert 3-4 times
- **Red:** Invert 5 times
- **Gold:** Invert 5 times
- **Green:** Invert 8-10 times
- **Purple:** Invert 8-10 times
- **Pink:** Invert 8-10 times
- **Dark blue:** Invert 8-10 times
- **Grey:** Invert 8-10 times



- ◆ When a large amount of blood sample is needed, an evacuated tube system with interchangeable glass tubes can be used to avoid multiple venipunctures.
- ◆ Evacuated tubes are commercially prepared with or without additives and with sufficient vacuum to draw a predetermined blood volume per tube.

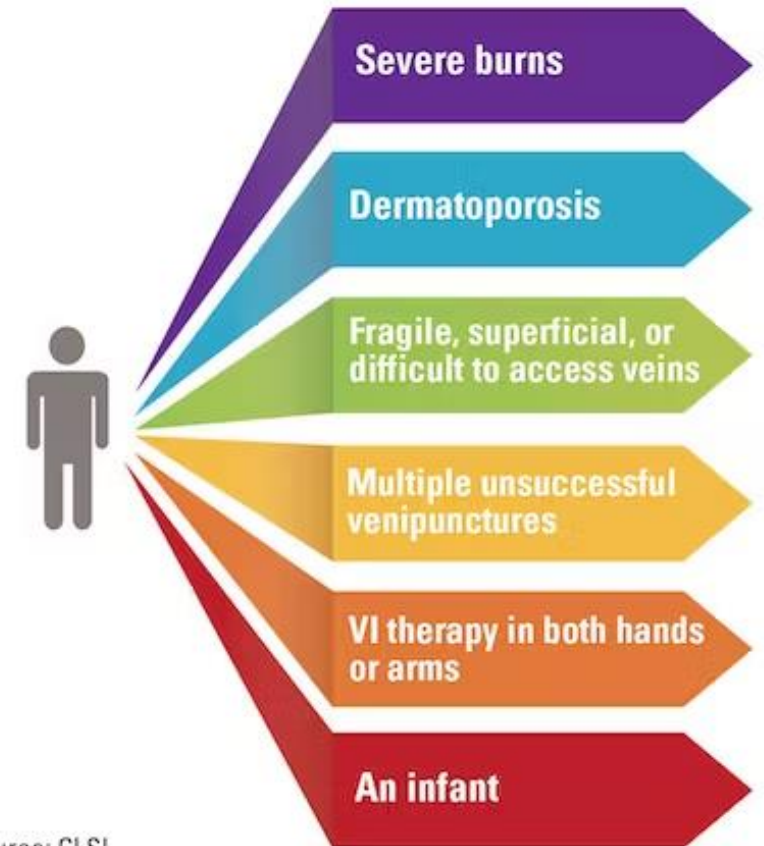




# Blood Samples:

- ❑ **Skin puncture** is an open collection technique in which the skin is punctured by a lancet and a small volume of blood is collected into a microdevice.
- ❑ Skin puncture blood is more like arterial blood than venous blood.
- ❑ In practice, it is used in situations in which:
  - (1) sample volume is limited (e.g., pediatric applications).
  - (2) repeated venipunctures have resulted in severe vein damage.
  - (3) patients have been burned or bandaged and veins therefore are unavailable for venipuncture.

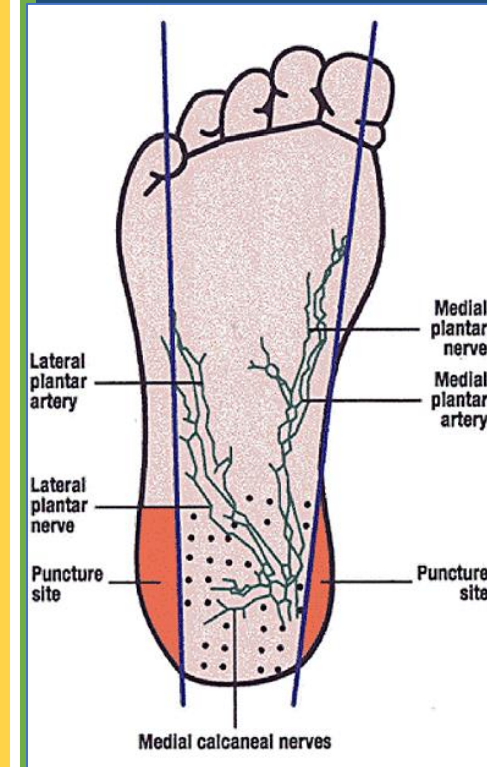
Capillary puncture collection may be a good choice if patients have one of the following characteristics:



Source: CLSI

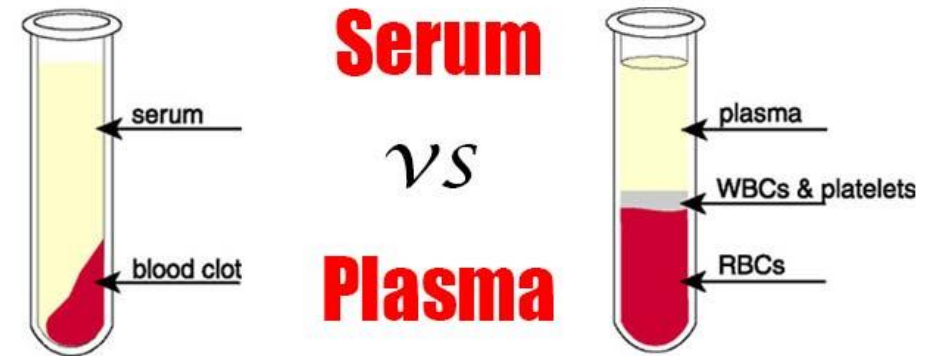
# Blood Samples:

- ❑ This technique is also commonly used when the sample is to be applied directly to a testing device in a point-of-care testing situation or to filter paper.
- ❑ It is a fairly painless procedure done on pediatric and neonatal patients.
- ❑ Example – Routine blood sugar testing by glucometer.
- ❑ It is most often performed on:
  - (1) the tip of a finger
  - (2) an earlobe
  - (3) the heel or big toe of infants.



# Blood Specimens:

- ◆ If blood is collected into a plain tube and allowed to clot, after centrifugation a **serum** specimen is obtained.
- ◆ If the blood is collected into a tube containing an anticoagulant such as heparin. When centrifuged, the supernatant is called **plasma**.



*Serum = Plasma – Clotting Factors*

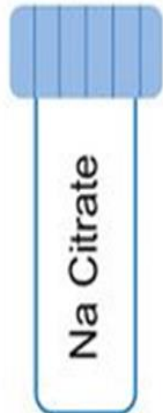
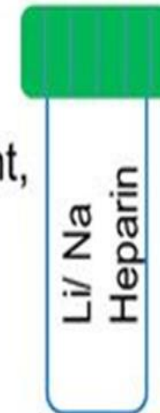
## SERUM

Clot for 30 min at room temperature, centrifuge 10 min at 4°C and 2,500 × g

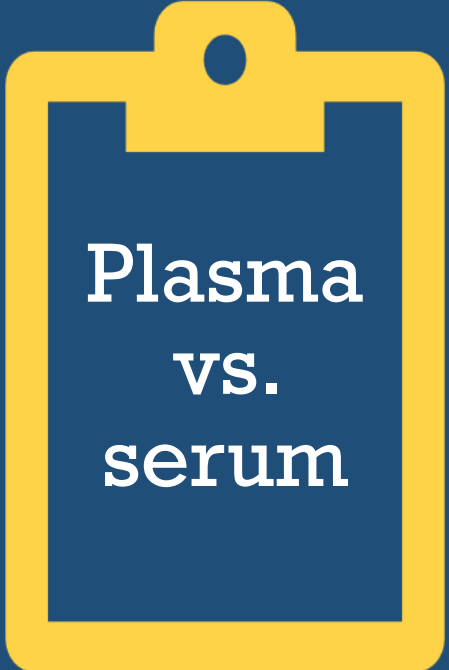


## PLASMA

Mix with anticoagulant, centrifuge 10 min at 4°C and 2,500 × g



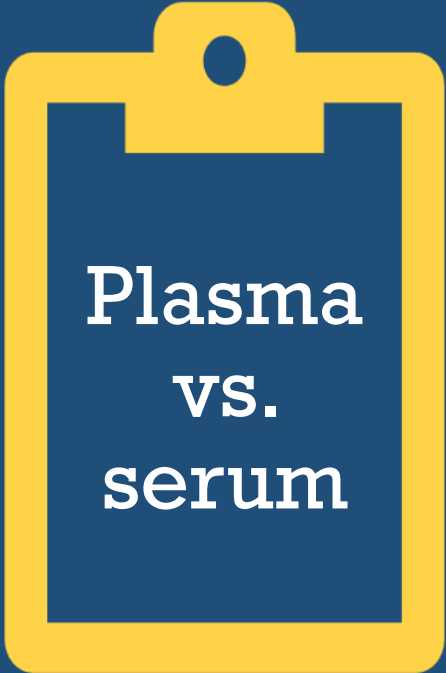
Characteristics	Serum	Plasma
<b>Definition</b>	Serum is the liquid part of the blood after the coagulation.	Plasma is a clear and yellowish fluid part of the blood.
<b>Composition</b>	Serum is the water fluid from blood without the clotting factors.	Plasma is the blood fluid that contains blood clotting agents.
<b>Volume</b>	Volume of serum is less in comparison to plasma.	Plasma is a clear yellow liquid which is 55% of total volume of blood.
<b>Isolation</b>	Serum is acquired from the process of spinning after clotting.	Plasma is acquired from the process of spinning before clotting.
<b>Isolation Procedure</b>	Serum is more difficult and time consuming to separate.	Plasma is easier and less time consuming to separate as compared with serum.



**Plasma  
vs.  
serum**



Characteristics	Serum	Plasma
<b>Use of Anticoagulants</b>	Serum does not need anticoagulants for separation.	Anticoagulants are necessary to separate plasma.
<b>Composition</b>	Serum contains proteins, electrolytes, antibodies, antigens and hormones.	Plasma is considered as the medium of blood in which RBCs (Red Blood Cells), WBC (White Blood Cells) and other components of blood are suspended.
<b>Composition (Antibodies)</b>	Serum contain antibodies and cross react with recipient antigen.	Blood plasma contains antibodies, a type of protein that can fight a substance considered foreign to the host body.
<b>Composition</b>	Serum contains proteins like albumin and globulins.	Plasma contains the clotting factors and water.
<b>Fibrinogen</b>	Absent	Present



## Plasma vs. serum

# Sampling Errors:

There are a number of potential errors that may contribute to the success or failure of the laboratory in providing the correct answers to the clinician's questions. Some of these problems arise when a clinician first obtains specimens from the patient.

## **I. Blood Sampling Technique:**

1. Difficulty in obtaining a blood specimen may lead to hemolysis with consequent release of potassium and other red cell constituents.
2. Prolonged stasis during venipuncture: Plasma water diffuses into the interstitial space and the serum or plasma sample obtained will be concentrated. Proteins and protein bound components of plasma, such as calcium or thyroxin, will be falsely elevated.
3. Insufficient specimen: It may not be possible for the laboratory to measure everything requested on a small volume.

# Sampling Errors:

## I. Blood Sampling Technique:

4. Errors in timing: The biggest source of error in the measurement of any analyte in a specimen is in the errors in timing.
5. Incorrect specimen container:
  - For many analyses the blood must be collected into a container with anticoagulant and/or preservative. For example, samples for glucose should be collected into a special container containing fluoride, which inhibits glycolysis; otherwise the time taken to deliver the sample to the laboratory can affect the result.
  - If a sample is collected into the wrong container, it should never be poured into another type of tube. For example, blood that has been exposed, even briefly, to EDTA (an anticoagulant) will have a markedly reduced calcium concentration, approaching zero, along with a false high potassium concentration. This is because EDTA is a chelator of calcium and is present as its potassium salt.

# Sampling Errors:

## I. Blood Sampling Technique:

6. Inappropriate sampling site: Blood samples should not be taken 'downstream' from an intravenous drip. It is not accepted for the laboratory to receive a blood glucose request on a specimen taken from the same arm into which 5% glucose is being infused.
7. Incorrect specimen storage: A blood sample stored overnight before being sent to the laboratory will show falsely high potassium, phosphate and red cell enzymes, such as lactate dehydrogenase, because of leakage into the extracellular fluid from the cells.



# Sampling Errors:

## **II. Biological Factors Affecting the Interpretation of Results:**

The differentiation between normal and abnormal results is affected by various physiological factors that must be considered when interpreting any given result. These include:

Stress &  
anxiety

Posture of the  
patient

Sex & Age

Effects of  
exercise

Medical  
history

Diet & Timing

# Urine Samples:

- Urine is an ultrafiltrate of the plasma. It can be used to evaluate and monitor body metabolic disease process, exposure to xenobiotic agents, mutagenicity, etc.
- Urine collection is a non invasive and readily obtainable. Clean and dry plastic or glass containers (50-3000 ml capacity) are used.
- Errors in timing in urine collection should be avoided because the biggest source of error in the measurement of any analyte in a 24-hour urine specimen is in the collection of an inaccurately timed volume of urine.
- If urine samples can not be handled within an hour, the samples should be kept in the fridge at around 4 °C for no longer than 24 hours to avoid multiplying of bacteria.
- However it is strongly recommended testing urine sample as soon as possible after collection.



# Types of Urine Specimens:

❖ 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:

1) **First morning urine specimen** (also known as an eight-hour specimen):

The urine is generally more concentrated in this type of specimen because it has been in the bladder for an extended period of time.

2) **Random urine specimen:** can be collected at any time, these specimens are usually satisfactory for routine screening and for cytology studies.

3) **Fractional urine collection:** A collection of urine taken during a few specified hours or from a specified quantity rather than from the entire amount voided during a day.

# Types of Urine Specimens:

- 4) **Timed urine collection:** Timed short-term specimens for 2 hour collections & Timed long-term specimens usually done over 12-24 hour period, this method allow day-to-day comparison.
- 5) **Midstream clean-catch specimens:** A mid-stream urine sample means you don't collect the first or last part of urine that comes out. This reduces the risk of the sample being contaminated with bacteria from: your hands, the skin around the urethra, the tube that carries urine out of the body. Used for urine culture and cytological analyses
- 6) **Double-voided specimen:** This refers to a urine specimen which is collected after first emptying the bladder and then waiting until another specimen can be collected. These specimens are more accurate for purposes of glucose testing.
- 7) **Urinary catheter specimen:** A urinary catheter is a flexible tube used to empty the bladder and collect urine in a drainage bag.

# Stool Samples:

- ❑ Stool tests can be a valuable diagnostic tool to assess digestive tract if there are signs and symptoms of gut infections as severe abdominal pain, bloating, frequent bowel movements, diarrhea, bloody diarrhea, or mucous in stool. The test includes a search for certain cells of interest, infectious markers and stool DNA.

## ❑ Stool Sample Collection:

- It should be collected in a sterile or clean, dry screw-top container.
- Freshly passed stool samples are taken and avoid specimens from a bedpan.
- Stool samples should be handed as soon as possible. If you can't hand the stool sample immediately, samples should store it in a fridge, but for no longer than 24 hours.
- Stool is stable for 1 hour at room temperature or 24 hours in the refrigerator.
- Specimen's greater than 96 hours (4 days) will be rejected.
- Stool collection for culture and sensitivity instructions leave at room temperature do not refrigerate.

# Stool Samples:

## What is the Best Time of the Day to Collect a Stool Sample?

Specimens are best obtained a few hours after the person has rested, between 9 p.m. & midnight, or in the morning immediately upon arising before bathing or bowel movement.

## What is Normal Stool Report?

Normal: The stool appears brown, soft, and well-formed in consistency. The stool does not contain blood, mucus, pus, undigested meat fibers, harmful bacteria, viruses, fungi, or parasites.

# Samples Transportation:

- Sample transportation requirements depends on the time, distance, climate, season, method, applicable regulations, type of specimen and markers to be assayed.
- Polyurethane boxes containing ice are used to ship and transport samples that require low temperature.
- For samples require very low temperature, liquid nitrogen container can be used. Liquid nitrogen containers are used for long term storage and transportation of biological specimens in the liquid nitrogen temperature zone ( $-196^{\circ}\text{C}$ ), such as blood samples, tissues, vaccines and viruses, etc.



Remember...  
Safety First!

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Thanks for your attention





# BLOOD PROTEINS TOTAL PROTEIN ALBUMIN

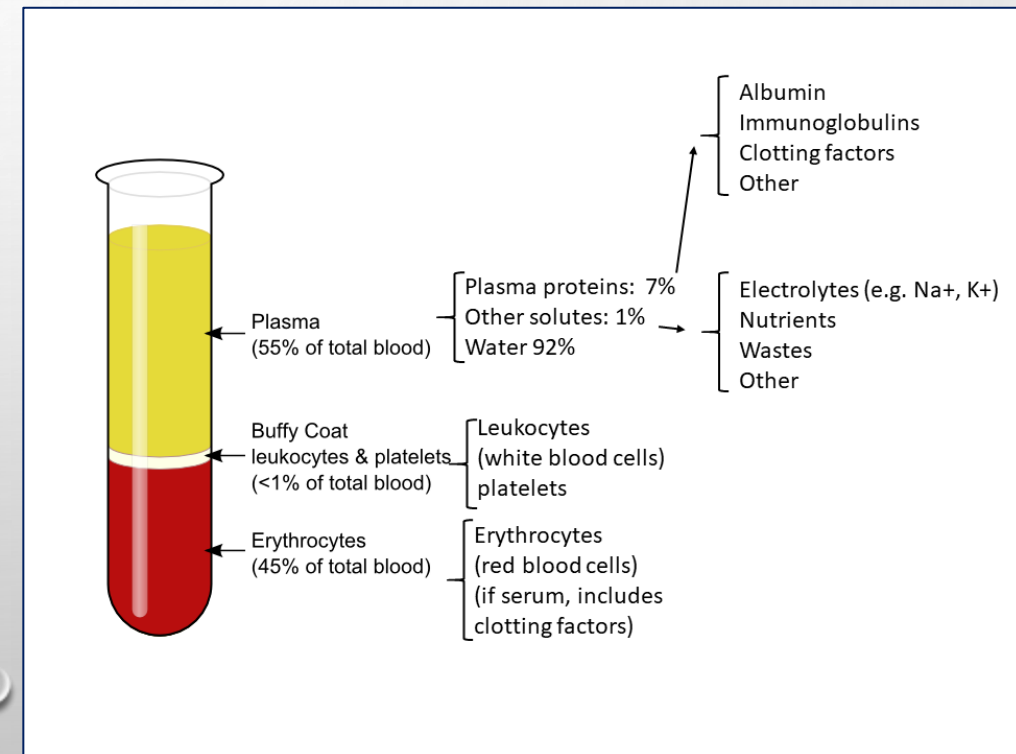
## Lab. 4

Dr. Ghaidaa S. Al-bana



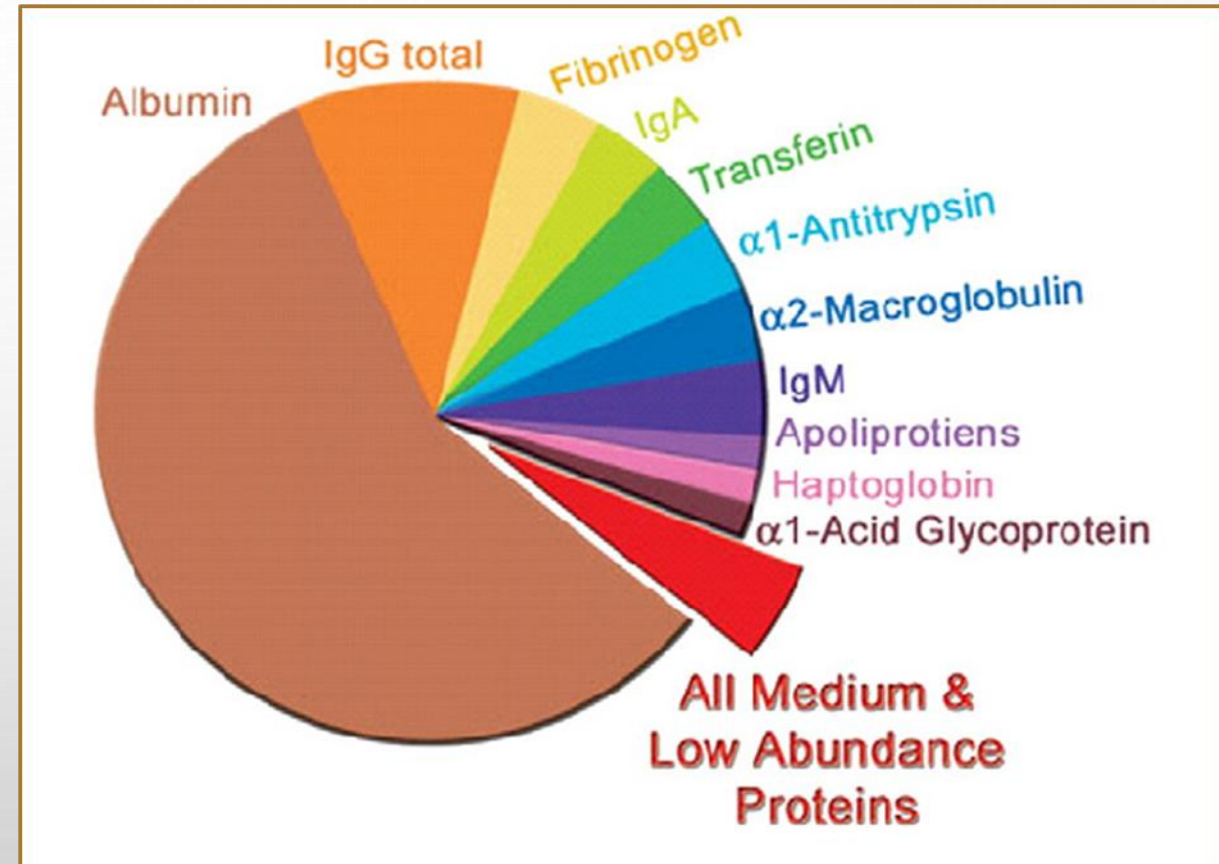
# INTRODUCTION

- Plasma consist of water, electrolytes, metabolites, nutrients, proteins and hormones.
- Concentrations of total proteins in human plasma is approximately 6.4- 8.3 g/dl which comprises the major part of solids of the plasma.
- Proteins dissolved in the plasma are complex mixture of both simple and conjugated proteins (glycoproteins & lipoproteins), each carry out a number of different functions.



# INTRODUCTION

- Over 300 proteins have been detected in plasma. The concentration of many of these are affected by pathological processes.
- The key role which plasma proteins play in bodily function, together with the relative ease of assaying them, makes their determination a valuable diagnostic tool as well as a way to monitor clinical progress.





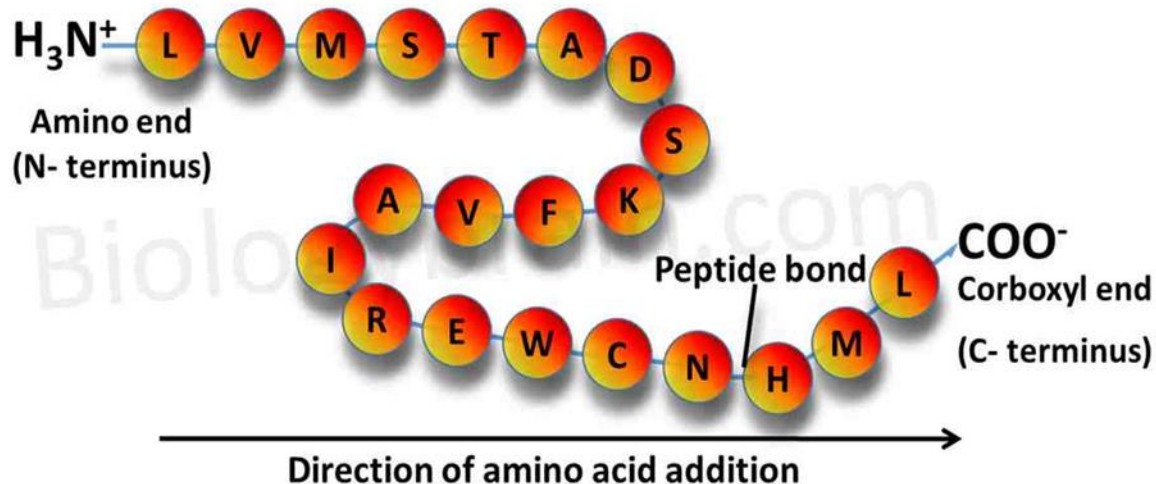
# INTRODUCTION

## Proteins common properties

- In spite of functional differences between the various serum proteins, they have certain common biophysical and biochemical properties. These include:

- I. A basic composition of carbon, hydrogen, nitrogen and oxygen.
- II. A backbone of covalent peptide bonds which join the amino acid units together.
- III. Absorption maxima in the ultraviolet region.
- IV. Based on these properties, laboratory methods have been developed to determine the concentration of proteins in serum.

### Protein primary structure and its important features



Protein primary structure represents the linear sequence (order) of amino acid units in the protein. This structure is stabilized by covalent peptide bonds, which provide metastability to the proteins and show higher level of stability even when heated at  $180^\circ C$ .

# SYNTHESIS & DEGRADATION OF PLASMA PROTEINS

- ❑ Almost all plasma proteins are both synthesized and destroyed in the liver, except for immunoglobulins which are synthesized in plasma cells & reticuloendothelial system.
- ❑ The liver synthesizes about 25g of plasma proteins every day, which accounts for nearly 50% of the total protein synthesis in the liver.
- ❑ While the site of synthesis of most plasma proteins is known with some certainty, the site of degradation is far from clear.
- ❑ Most proteins are degraded by most tissues throughout the body. Most proteins are degraded after being taken up by cells within the body.
- ❑ Liver, GIT, muscle & kidneys being the main sites of protein catabolism.
- ❑ The control of protein synthesis and degradation is quite complex & several factors may influence the process such as dietary status, circulating plasma level (feedback), hormonal & neural.

# FUNCTIONS OF PLASMA PROTEINS

- ✓ **Acid-base regulation:** plasma protein being amphoteric can act as a buffer to maintain the balance between the acid-base of blood and other bodily fluids.
- ✓ **Colloidal osmotic pressure:** aka oncotic pressure is maintained by plasma proteins, which are essential for the distribution of water in blood vessels and interstitial spaces.
- ✓ **Blood clotting:** plasma proteins such as fibrinogen, prothrombin, and other blood clotting factors are present in an inactive form in plasma. During the injury, they get activated and help in blood clotting.
- ✓ **Humoral immunity:** B lymphocytes form immunoglobulins which are present in plasma and provide immunity against pathogens.

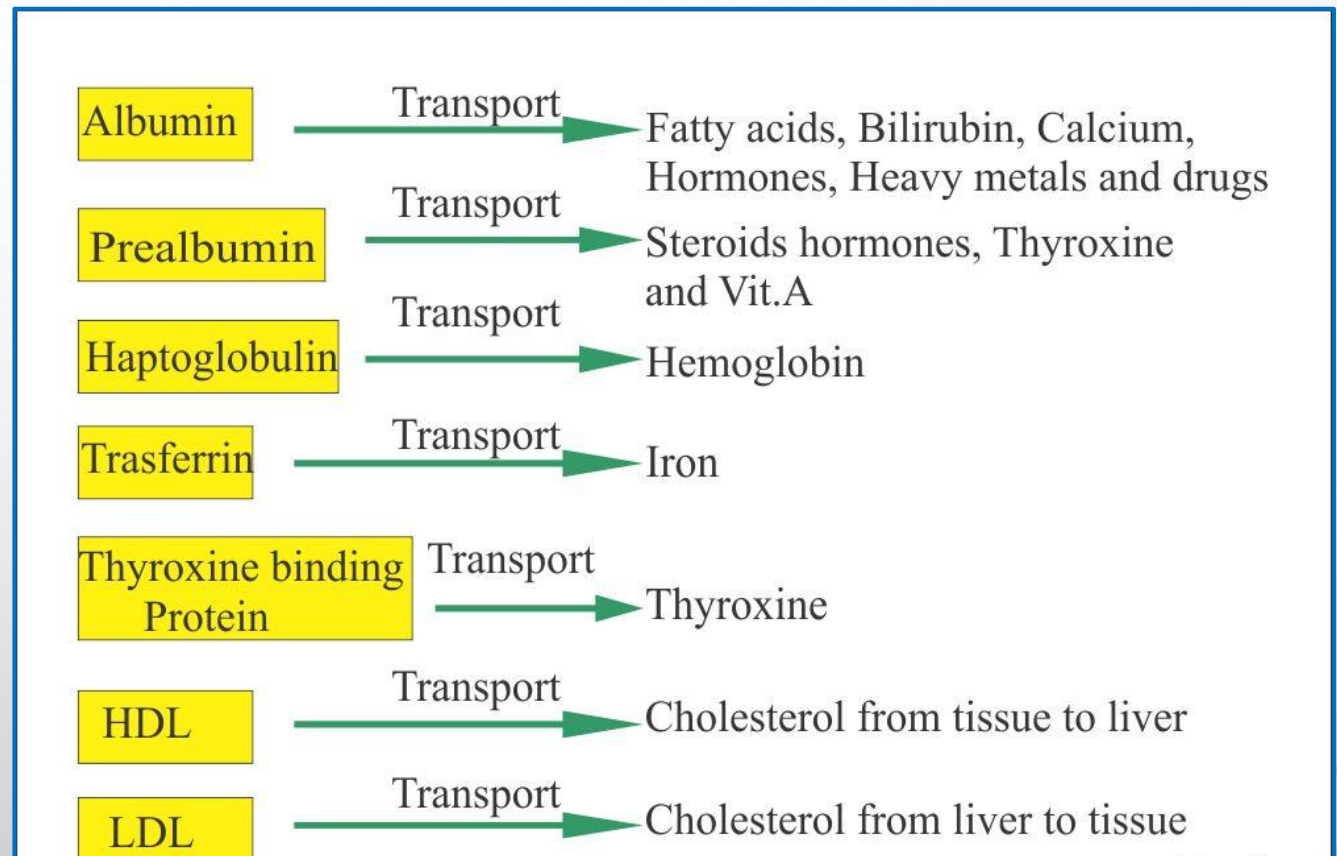
# FUNCTIONS OF PLASMA PROTEINS

- ✓ **Provide nutrition:** plasma proteins are simple proteins that contain amino acid and thus provide endogenous source of energy.
- ✓ **Maintain viscosity in blood:** the presence of globulins and fibrinogens in plasma helps in maintaining the viscosity of blood, which is important for maintaining normal blood pressure.
- ✓ **Storage of enzymes:** lipase, amylase, and transaminase are enzymes stored in plasma in small amounts. Changes in their concentration in plasma can be a biomarker for the disease.
- ✓ **Reserve proteins:** plasma proteins are converted to amino acids during fasting and carried to tissues by the circulation of blood and use to synthesize tissue protein.



# FUNCTIONS OF PLASMA PROTEINS

✓ **Transport of substances:**  
transport of large molecules from the blood to tissue is carried out by albumin and globulins.





# FUNCTIONS OF PLASMA PROTEINS

## Proteins and their functions

Type of protein	Quantity	Site of formation	Functions
Albumin	60%	Liver	Maintain blood osmotic pressure
Globulins	36%		
Alpha Globulin		Liver	Transport lipids, fats, and fat-soluble vitamins.
Beta Globulins		Liver	Transport lipids, fats, and fat-soluble vitamins.
Gamma Globulins		Lymphatic system	Take part in the immune system
Fibrinogen	4%	Liver	Take part in blood coagulation

# Clinical Significance

- In very general terms, variations in plasma protein concentrations can be due to any of three changes:



The volume of plasma water  $\Rightarrow$   
change volume of distribution



Rate of protein synthesis  $\Rightarrow$   
changes in protein concentration



Rate of protein catabolism  $\Rightarrow$   
changes in protein concentration

## Hypoproteinemia

- I. Malnutrition and/or malabsorption.
- II. Excessive protein loss as in renal disease, GI leakage.
- III. excessive bleeding, severe burns.
- IV. Increased protein catabolism, like in malignancies and inflammation
- V. Decrease protein synthesis like liver diseases
- VI. Water intoxication.
- VII. Massive IV infusions.

## Hyperproteinemia

- I. Dehydration due to inadequate water intake or excessive water loss due to vomiting, diarrhea.
  - II. Monoclonal (gammopathy) increases.
  - III. Polyclonal increase.
- Only disorders affecting the concentration of albumin and/or the immunoglobulins will give rise to abnormal total protein levels.
  - Other serum proteins are never present in high enough concentrations for changes to have a significant overall effect.

# DETERMINATION OF TOTAL PLASMA PROTEINS UTILIZING BIURET METHOD

## ❖ Principle:

- Peptide bonds ( $\text{-C- N-}$ ) of proteins react with tartrate-complexed cupric ions in alkaline solutions to form a violet colored product.
- In a positive test, a copper(II) ion is reduced to copper(I), which forms a complex with the nitrogen and carbon of the peptide bonds in an alkaline solution.
- A violet color indicates the presence of proteins.
- The intensity of the color, and hence the absorption is directly proportional to the protein concentration, and can be determined spectrophotometrically at 550 nm.



# DETERMINATION OF TOTAL PLASMA PROTEINS UTILIZING BIURET METHOD

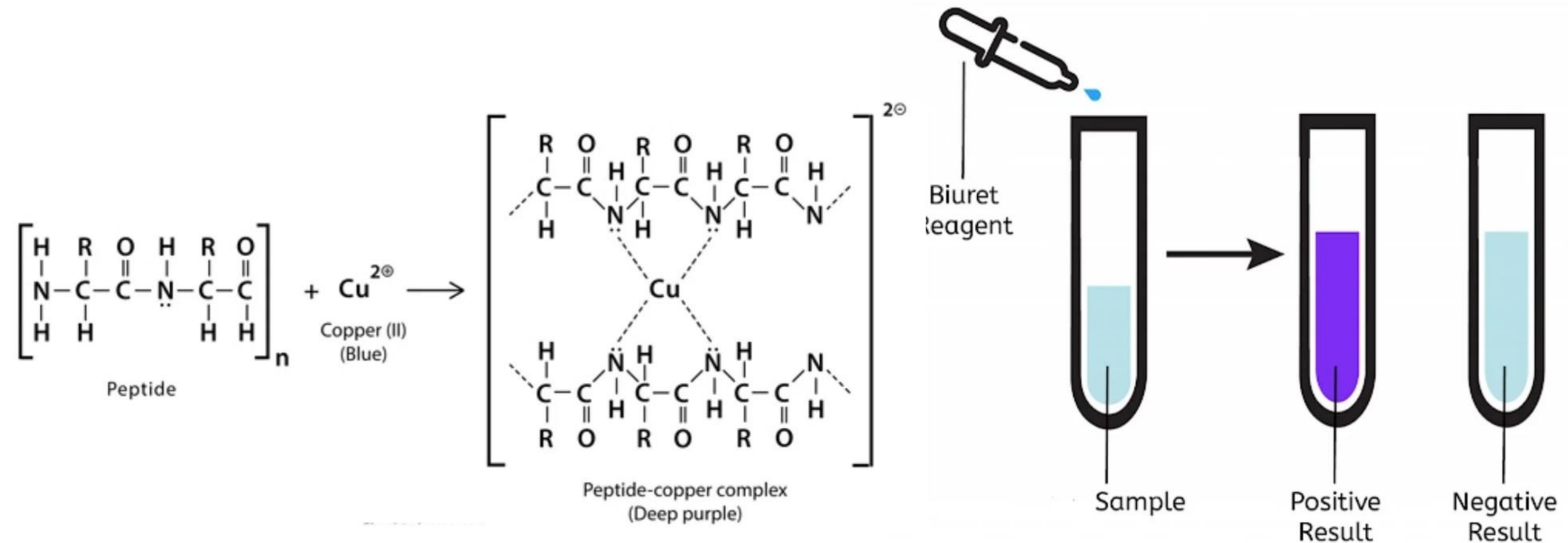
## ❖ Reagents:

- ✓ Biuret reagent is a type of solution which is composed of sodium hydroxide (NaOH), hydrated copper (II) sulfate ( $\text{CuSO}_4$ ), together with potassium -sodium tartrate ( $\text{KNaC}_4\text{H}_4\text{O}_6$ ).
- ✓ Sodium hydroxide provides the alkaline medium and potassium -sodium tartrate is added to chelate and thus stabilize the cupric ions in the solution or to maintain their solubility in alkaline solution.
- ✓ Bovine albumin is used as a standard.

## ❖ Specimen:

- ✓ Serum and plasma may be used, and all usually yield comparable results, though, because of the presence of fibrinogen, plasma levels for total protein are 2 to 4 g/L higher than serum levels.
- ✓ A fasting specimen is not required but may be desirable to decrease lipemia.
- ✓ Total protein is stable in serum and plasma for:
  - 1 week at room temperature.
  - at least 2 months at  $-20^\circ \text{C}$ .

# Biuret Test for Protein





# DETERMINATION OF TOTAL PLASMA PROTEINS UTILIZING BIURET METHOD

## ❖ Procedure:

- Take three test tubes that are clean and dry.
- In each test tube, add 1-2 ml of the test solution, standard, and deionized water.
- Add 1 to 2 ml of Biuret reagent to each test tube.
- Shake the ingredients vigorously and let it stand for 5 minutes at room temperature.
- Observe any changes in color.
- Measure the absorbance of the sample & the standard at 550 nm against reagent blank.

## ❖ Calculation:

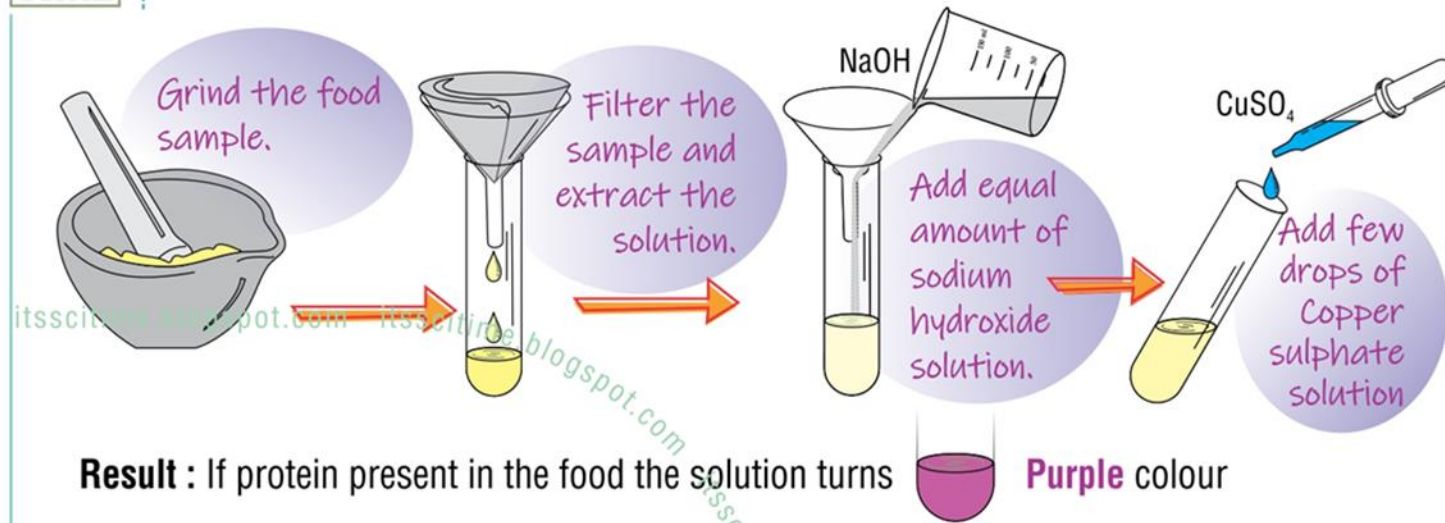
$$\text{Serum total protein concentration} = \frac{\text{Abs (sample)}}{\text{Abs (standard)}} \times \text{standard concentration} \dots$$

❖ **Reference range** for total proteins is 66.6 to 81.4 g/L

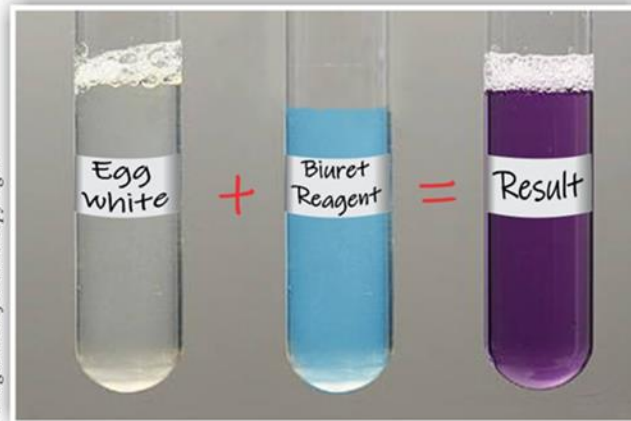
❖ Results for males are approximately 1 g/L higher than results for females; this difference is probably not of clinical significance.



## FOOD TESTS - Protein Test (Biurete Test)



**Real colour change...**

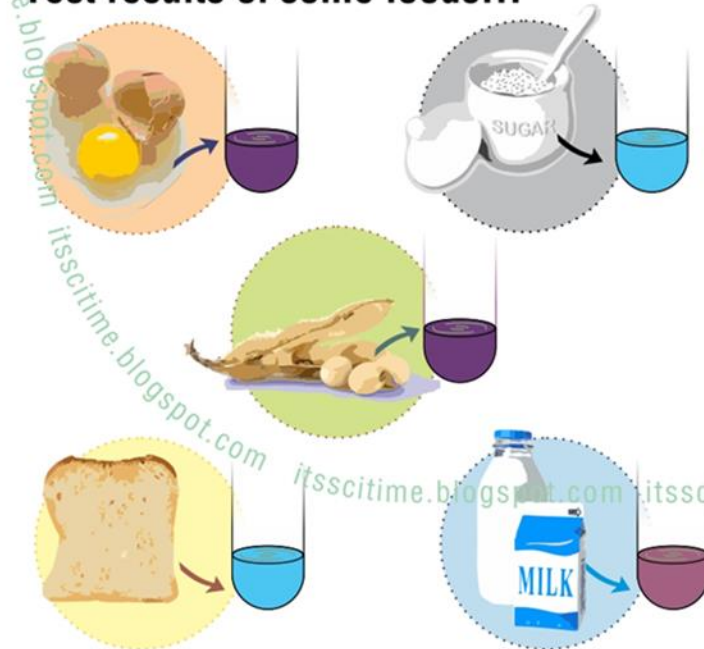


\* Images is subjected to copy right.

**What is Biuret reagent?**

Biuret Reagent is an aqueous solution of potassium sodium tartrate treated with cupric sulfate and sodium hydroxide.

**Test results of some foods...**

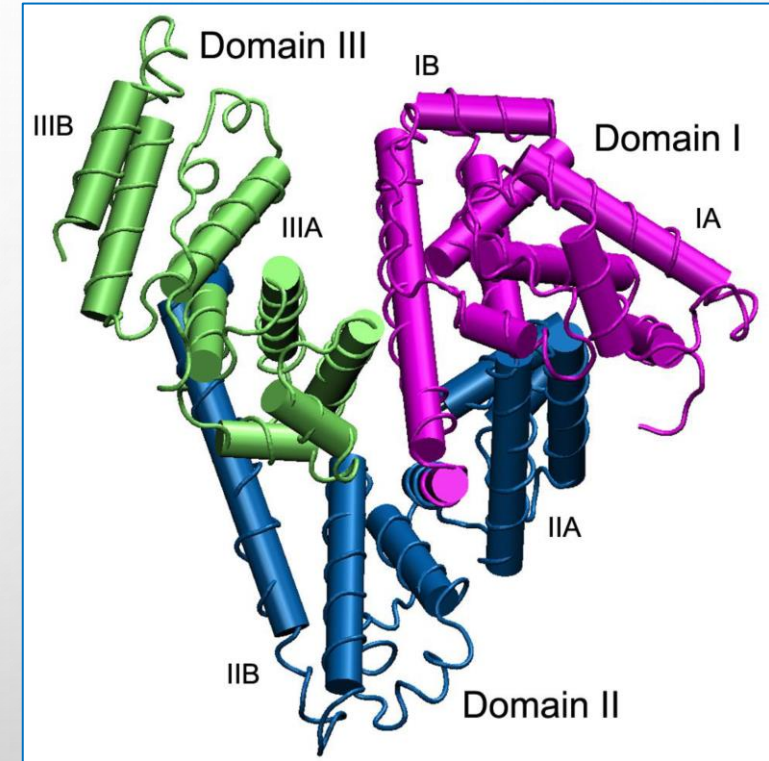


## INTERFERING FACTORS

- Prolonged application of a tourniquet can increase both albumin & globulin concentrations.
- Sampling of peripheral venous blood proximal to an IV infusion site can result in low protein levels
- Drugs that may increase protein levels include: anabolic steroids, androgens, corticosteroids, dextran, growth hormone, insulin, phenazopyridine & progesterone.
- Drugs that may decrease plasma protein levels include: estrogens, hepatotoxic drugs, oral contraceptives & ammonium ions.

# SERUM ALBUMIN

- Albumin (69 KDa) is the major plasma protein (60%) which is unique in being carbohydrate free.
- It is a single polypeptide chain having 585 amino acid with 17 disulfide bonds.
- It is synthesized in the liver at a rate of 10- 12 g/day which represents 25% of the total hepatic protein synthesis.
- For this reason, measurement of serum albumin concentration is a useful tool for the assessment of liver functions.
- Albumin has a half-life of 17-20 days in the circulation.





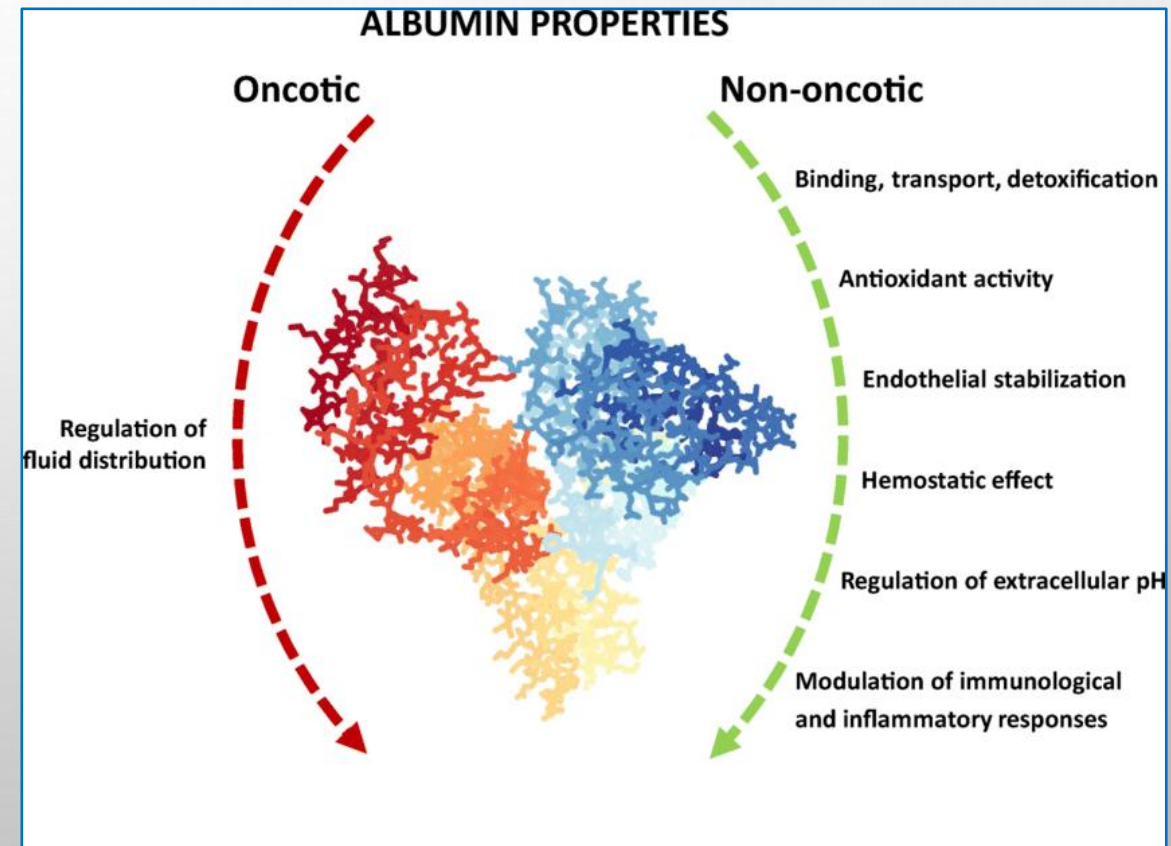
# FUNCTIONS OF ALBUMIN

**1. Transport:** albumin binds & transports many diverse molecules serving as a low- specificity transporter protein, those include:

- Free fatty acids: albumin binds to FFA released by the adipose tissues & facilitate their transport to other tissues.
- Metal ions: such as calcium & copper.
- Bilirubin: this decrease the toxic side effects of unconjugated bilirubin.
- Bile acid: albumin carries the bile acids that are recycled from the intestine to the liver in the hepatic portal vein.

**2. Source of endogenous amino acids.**

**3. Maintaining acid- base balance.**

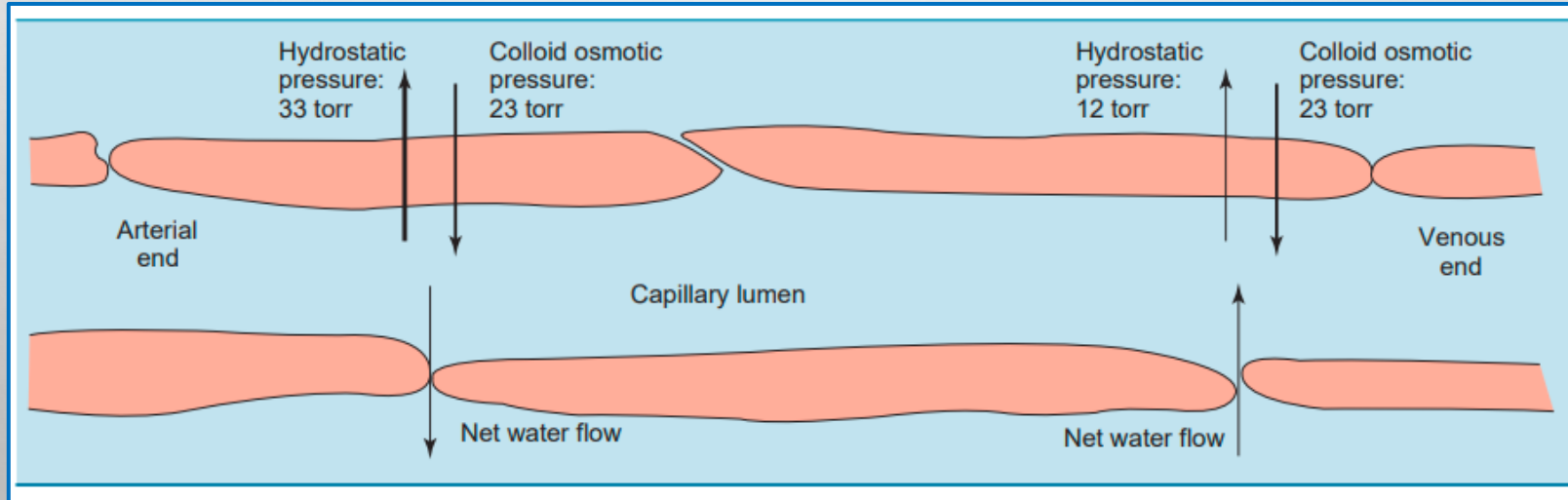


# FUNCTIONS OF ALBUMIN

## 4. Maintain plasma oncotic pressure (colloid osmotic pressure):

Although albumin accounts for only 60% of the total plasma protein, it provides 80% of the colloid osmotic pressure. This is because the colloid osmotic pressure depends on the amount of water and electrolytes that a protein attracts to its surface, and albumin is one of the most hydrophilic plasma proteins.

The colloid osmotic pressure is necessary to prevent edema. The hydrostatic pressure of the blood forces fluid from the capillaries into the interstitial spaces, and the colloid osmotic pressure of the plasma proteins is required to pull the fluid back into the capillaries.



# CLINICAL SIGNIFICANCE

- ❑ The normal range of serum albumin is 3.5- 5.2 g/dl, while that for albumin/globulin ratio is 1.3- 1.8.
- ❑ the main factors that effect normal albumin levels are:
  - The volume of its distribution.
  - The of both its synthesis and catabolism.
- ❑ in most disease processes a change in more than one of these factors is operating.
- ❑ Hyperalbuminemia: no clinical conditions are known to increase liver production of albumin & the only cause that may increase plasma albumin level is dehydration.

# CLINICAL SIGNIFICANCE

## □ Hypoalbuminemia:

- Decreased albumin synthesis: e.g. liver diseases, malnutrition, malnutrition, following acute phase response.
- Increased albumin loss: as in nephrotic syndrome, protein losing enteropathy & burn.
- Increased albumin catabolism: e.g. Cushing's syndrome, tumors, thyrotoxicosis.
- Tissue damage or inflammation leading to increase albumin breakdown.

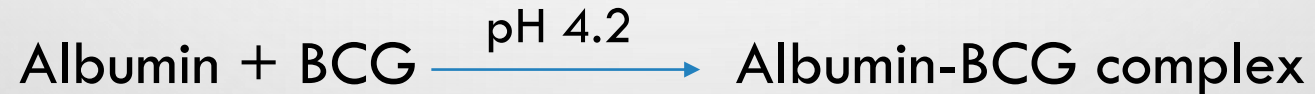


# QUANTITATIVE DETERMINATION OF ALBUMIN IN SERUM

**Bromocresol green (BCG)** albumin assay is designed to measure albumin directly in biological samples without any pretreatment.

## □ Principle:

- Albumin (pI 4.9) at pH 4.2 is sufficiently cationic to bind the **anionic dye bromocresol green (BCG)** to form a **blue-green** colored complex.



- The intensity of the blue-green color is directly proportional to albumin concentration in the specimen.
- It is determined by measuring the increase in absorbance at 620 - 630 nm.

# Reagents compositions

## Vial R1 (Bromocresol green)

- Succinic acid 83 mmol/L
- Bromocresol green (BCG) 167  $\mu$ mol/L
- Sodium hydroxide 50 mmol/L
- Polyoxyethylene monolauryl ether 1.00 g/L
- Preservative

## Vial R2 (Standard)

- Bovine albumin 5 g/dl

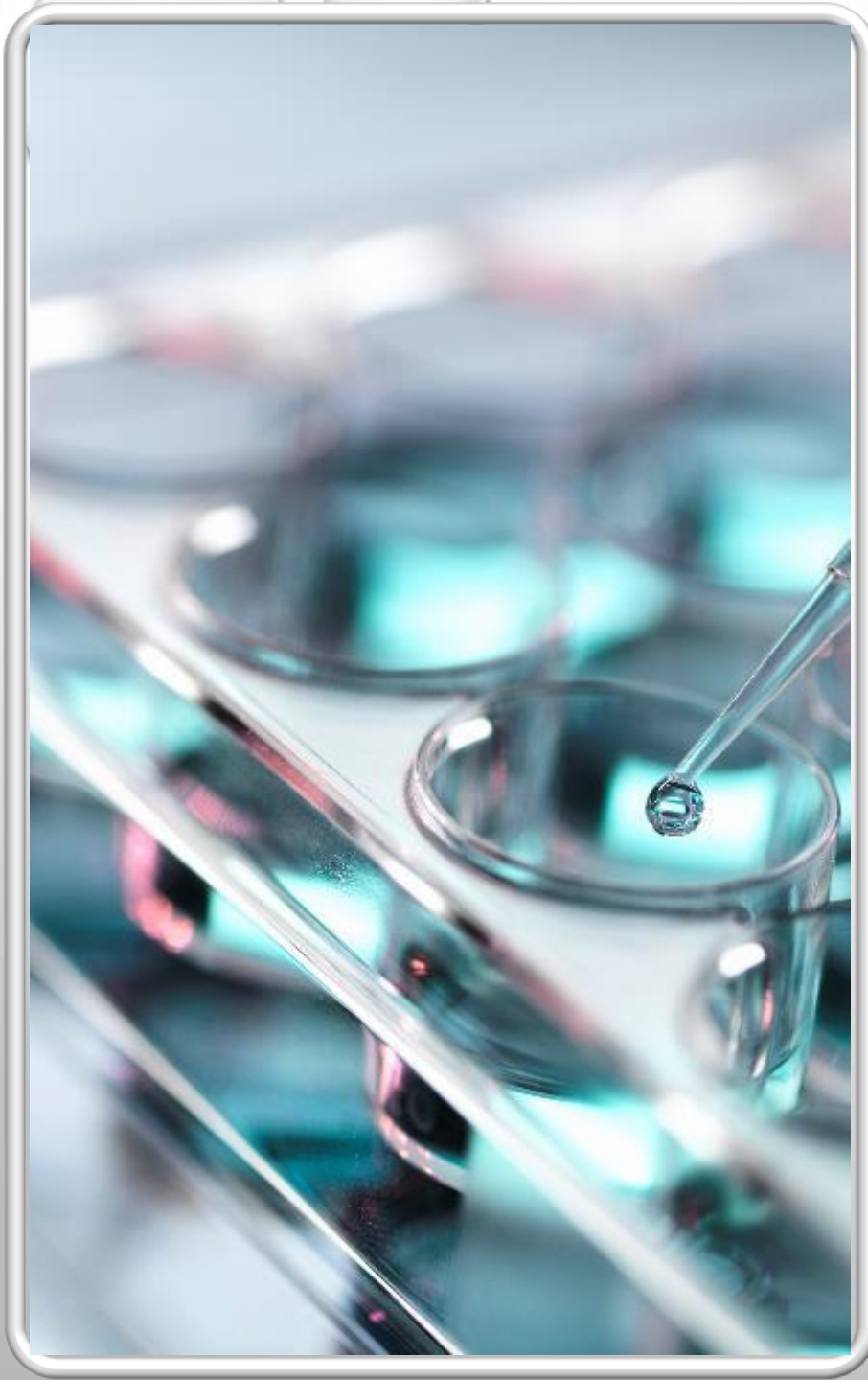


## Procedure

Pipette into well Identified test tubes	Blank	Standard	Assay
<b>Demineralized water</b>	10 $\mu$ l	.....	.....
<b>Standard</b>	.....	10 $\mu$ l	.....
<b>Sample</b>	.....	.....	10 $\mu$ l
<b>Reagent</b>	2.0 ml	2.0 ml	2.0 ml

Mix well. Record absorbance at 630 nm (620-640) within 3 minutes against reagent blank or better after exactly 1 minute.





THANK YOU FOR  
YOUR ATTENTION



# **Serum Calcium** **Inorganic Phosphate**

## **Lab. 5**

**Dr. Ghaidaa S. Al- Bana**

# Serum Calcium

- ❑ It would be very difficult to name a physiologic process that does not depend, in one way or another, on calcium.
- ❑ It is critical to maintain blood calcium concentrations within a tight normal range.
- ❑ Deviations above or below the normal range frequently lead to serious diseases.



# Endocrine Control of Calcium Homeostasis

- ❑ **Hypocalcemia** refers to low blood calcium concentration. Clinical signs of this disorder reflect increased neuromuscular excitability and include muscle spasms, tetany and cardiac dysfunction.
- ❑ **Hypercalcemia** indicates a concentration of blood calcium higher than normal. The normal concentration of calcium and phosphate in blood and extracellular fluid is near the saturation point; elevations can lead to diffuse precipitation of calcium phosphate in tissues, leading to widespread organ dysfunction and damage.
- ❑ Preventing hypercalcemia and hypocalcemia is largely the result of robust endocrine control systems.





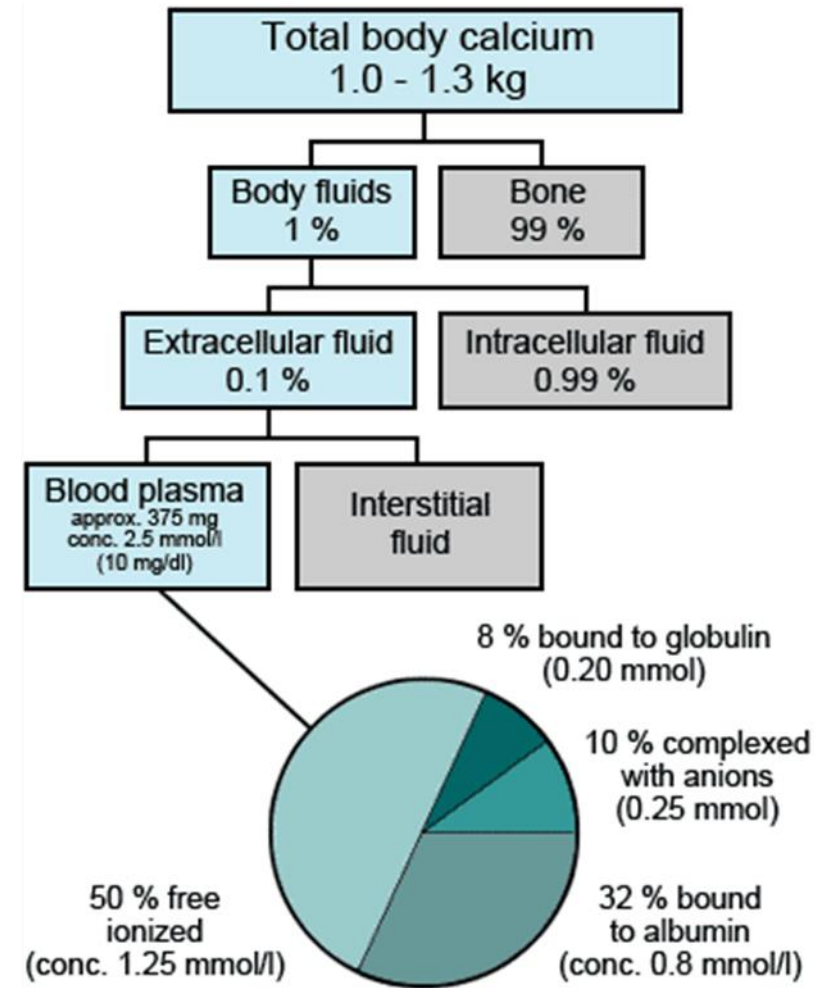
# Body Distribution of Calcium

- ❑ The adult human body contains 1-1.3 kg of calcium. Almost all (99%) of this is contained within bones and teeth. The remaining 1% is distributed between soft-tissue cells and extracellular fluid (i.e. interstitial fluid and blood plasma). Just 8.7 mmol (350 mg) calcium circulates in blood plasma at a concentration of around 2.5 mmol/L (10 mg/dL).
- ❑ Of this 350 mg around 40% is bound to protein (predominantly albumin, but also globulins) and 10% is complexed with a range of anions (bicarbonate, lactate, phosphate, etc). The remaining 50% circulates as "free" ionized calcium ( $\text{Ca}^{2+}$ ) at a concentration of around 1.25 mmol/L.



# Body Distribution of Calcium

- ❑ The three fractions of calcium present in blood plasma are in equilibrium, but crucially only the ionized calcium fraction is physiologically active. A small proportion ( $\gg 1\%$ ) of the calcium in bone is readily exchangeable with plasma.



# Fluxes of Calcium and Phosphate

Maintaining constant concentrations of calcium in blood requires frequent adjustments, which can be described as fluxes of calcium between blood and other body compartments. Three organs participate in supplying calcium to blood and removing it from blood when necessary:

- ❖ **The small intestine** is the site where dietary calcium is absorbed. Importantly, efficient absorption of calcium in the small intestine is dependent on expression of a calcium-binding protein in epithelial cells.
- ❖ **Bone** serves as a vast reservoir of calcium. Stimulating net resorption of bone mineral releases calcium and phosphate into blood, and suppressing this effect allows calcium to be deposited in bone.
- ❖ **The kidney** is critically important in calcium homeostasis. Under normal blood calcium concentrations, almost all of the calcium that enters glomerular filtrate is reabsorbed from the tubular system back into blood, which preserves blood calcium levels. If tubular reabsorption of calcium decreases, calcium is lost by excretion into urine.

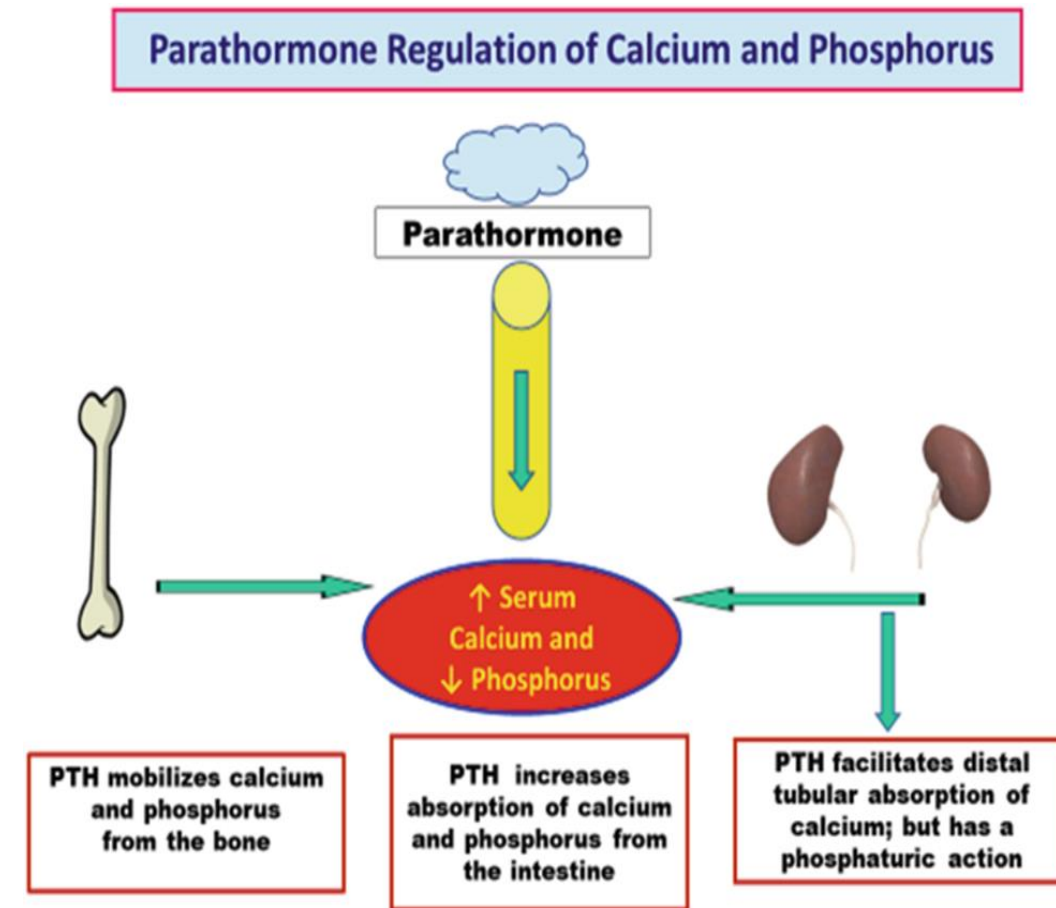


Maintaining normal blood calcium and phosphorus concentrations is managed through the concerted action of **three hormones** that control fluxes of calcium in and out of blood and extracellular fluid:

**1. Parathyroid hormone** serves to increase blood concentrations of calcium. Mechanistically, parathyroid hormone preserves blood calcium by several major effects:

- I. **Stimulates production** of the biologically-active form of vitamin D within the kidney.
- II. **Facilitates mobilization** of calcium and phosphate from bone. To prevent detrimental increases in phosphate, parathyroid hormone also has a potent effect on the kidney to eliminate phosphate (phosphaturic effect).

## Hormonal Control Systems

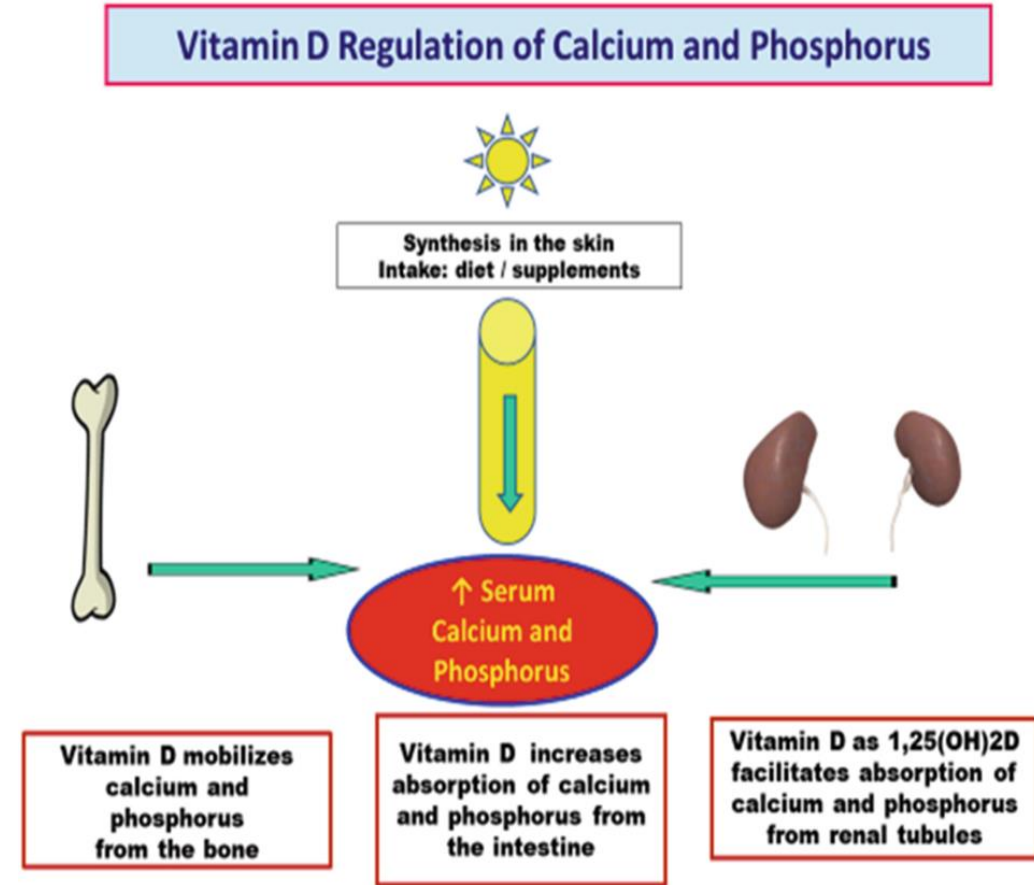


# Hormonal Control Systems

III. Maximizes tubular reabsorption of calcium within the kidney. This activity results in minimal losses of calcium in urine.

2. Vitamin D acts also to increase blood concentrations of calcium.

- It is generated through the activity of parathyroid hormone within the kidney.
- Far and away the most important effect of vitamin D is to facilitate absorption of calcium from the small intestine.
- In concert with parathyroid hormone, vitamin D also enhances fluxes of calcium out of bone.



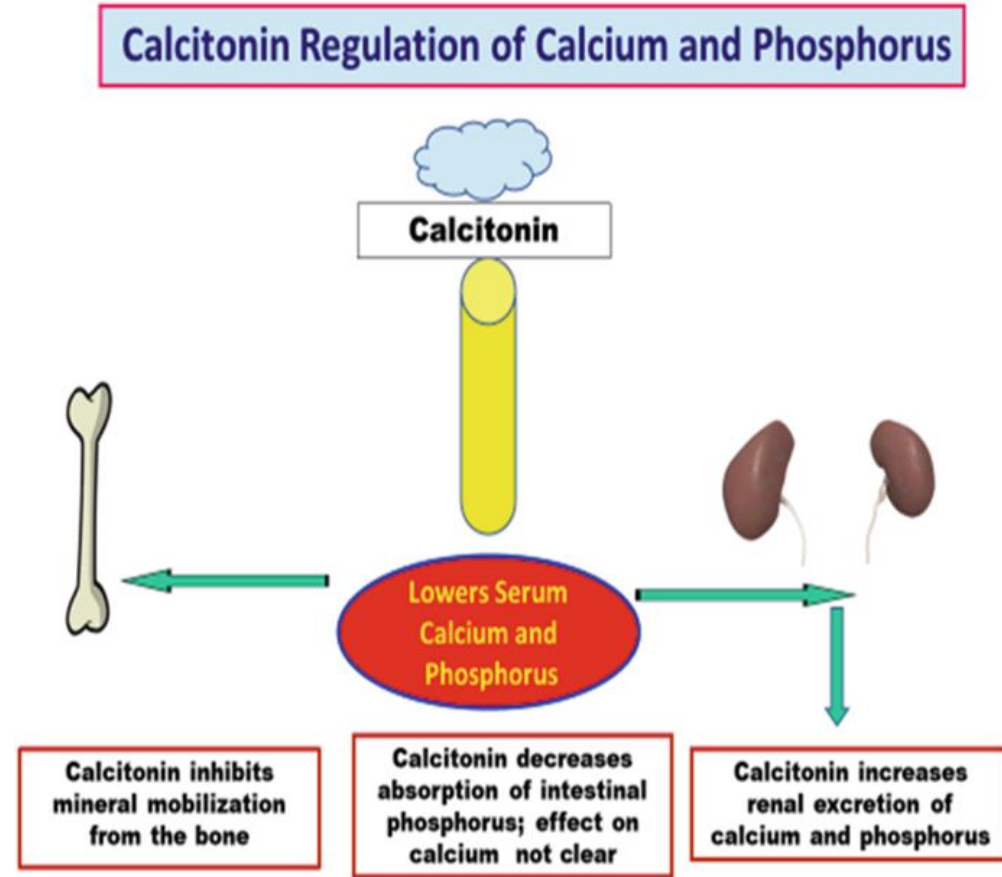


# Hormonal Control Systems

**3. Calcitonin** is a hormone that functions to reduce blood calcium levels. It is secreted in response to hypercalcemia and has at least two effects:

- I. **Suppression of renal tubular reabsorption of calcium.** In other words, calcitonin enhances excretion of calcium into urine.
- II. **Inhibition of bone resorption,** which would minimize fluxes of calcium from bone into blood.

Although calcitonin has significant calcium-lowering effects in some species, it appears to have a minimal influence on blood calcium levels in humans.



# Assessing Calcium Status

## The rationale for measuring ionized rather than total plasma calcium concentration

- ❖ For over 50 years the most common method of assessing calcium status has been to measure concentration of total calcium (i.e. the sum of protein-bound, complexed and free ionized calcium) in plasma or serum utilizing the colorimetric methods.
- ❖ The clinical validity of total calcium measurement is based on the assumption that total calcium concentration accurately reflects ionized calcium activity (the fraction that is clinically significant). Whilst that is the case in healthy individuals, it is not necessarily the case in some sick individuals.





# Assessing Calcium Status

- ❑ The two main clinical situations in which total calcium concentration does not sufficiently and accurately reflect ionized calcium activity are:
  - those in which the patient's serum protein concentration is abnormal.
  - those in which the patient is suffering disturbance of the acid-base balance.
- ❑ The significance of **serum protein concentration** lies in the observation that the amount of calcium bound by serum proteins is directly proportional to protein concentration.
- ❑ The important point is that despite an increase or decrease in measured total calcium concentration, ionized calcium concentration, the physiologically and clinically important parameter, remains essentially unchanged.



# Assessing Calcium Status

- ❑ Since most of the protein-bound calcium in serum is bound to albumin, it is **the change in serum albumin concentration that is most significant in affecting total calcium concentration**. Interpretation of total calcium results should always include due concern of serum albumin concentration.
- ❑ To lessen the effect of abnormal serum protein concentration on total calcium measurement, a number of formulae have been devised for estimation of "corrected" total calcium concentration from measured total calcium and either serum albumin or protein concentration.
- ❑ One of the most widely used ones in the US is the so-called "modified Orrell correction":

Corrected calcium (mg/dL) = measured total calcium (mg/dL) + 0.8 (4 – serum Alb g/dL)



# Assessing Calcium Status

- ❑ **Blood pH** is a major determinant of the proportion of total calcium that is bound to protein, principally because hydrogen ions compete with calcium ions for protein binding sites.
- ❑ A decreased pH (acidosis) is associated with decreased calcium binding and therefore increased proportion of total calcium in the ionized state.
- ❑ To give some idea of the magnitude of this effect, each 0.1 decrease in pH results in a 0.05 mmol/L increase in serum ionized calcium concentration. By the same mechanism, raised blood pH (alkalosis) causes reduction in serum ionized calcium concentration.
- ❑ Since this phenomenon is merely a shift of calcium from one fraction to another, ***total*** calcium concentration is not affected.



# Assessing Calcium Status

- ❑ Although "corrected" total calcium reflects ionized calcium more accurately than uncorrected total calcium in patients with abnormal serum protein concentration, none of the correction formulae are entirely reliable for all patients.
- ❑ The limitations of the correction formulae:
  - It failed to accurately classify calcium status (hypocalcemia, normocalcemia or hypercalcemia) in 38 % of 110 intensive care patients.
  - Serum/plasma total calcium does not accurately reflect ionized calcium activity in patients suffering disturbance of acid-base.



# General Principles of Current Testing Methods

## Total Calcium

- The field method for total serum calcium depends on reaction with a chelating compound that results in color development that can be measured optically.
- For example, calcium reacts with o-cresolphthalein complex one (o-CPC) under alkaline conditions to form a violet-colored complex that is monitored at 600 nm.

## Free or Ionized Calcium

- Ionized (free) calcium, is determined via electrochemical methods using a calcium ion-selective electrode (ISE).
- The presence of calcium ions creates an electrical potential relative to a reference electrode, which is measured and ultimately converted to an  $iCa^{2+}$  concentration.
- The free-calcium concentration is proportional to the potential difference between the indicator electrode and the reference electrode.



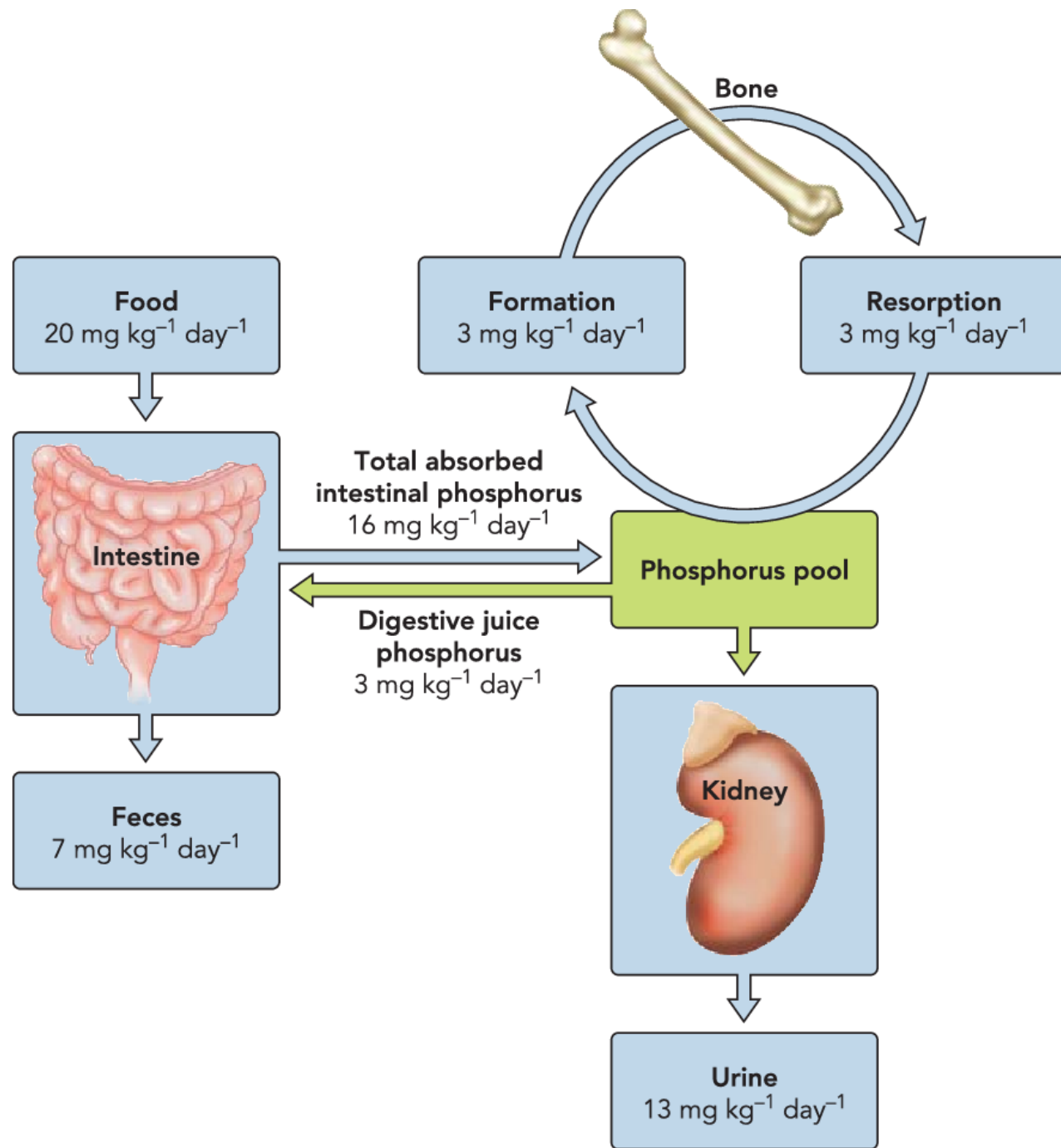


# Inorganic Phosphate

## Introduction:

- ☐ Phosphate is involved in many critically important biochemical processes including:
  - ✓ energy metabolism
  - ✓ nucleic acid metabolism
  - ✓ cell signaling
  - ✓ bone formation
  - ✓ maintenance of acid–base balance.
- ☐ The only source of phosphate is external, from daily dietary food, which has to travel in the gut and become available for intestinal absorption.







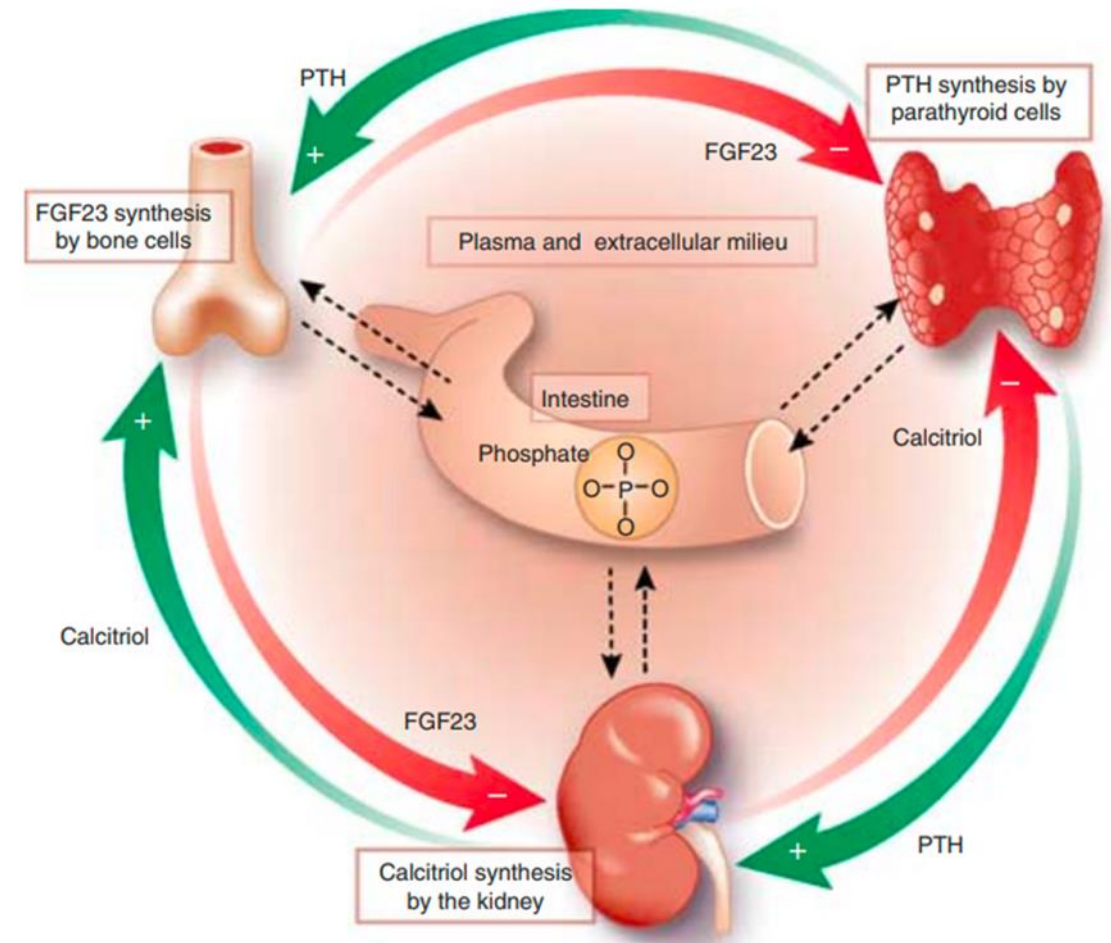
# Introduction

- Human body contains both inorganic and organic phosphate. **Organic phosphate esters** are found primarily within cells. **Inorganic phosphate** is a major component of hydroxyapatite in bone, thereby playing an important role in the structural support of the body and providing phosphate for extracellular and intracellular pools.
- Approximately 10% of the phosphate in serum is protein bound, 35% complexed with calcium, and magnesium; the remainder is free.
- In plasma, inorganic phosphate exists as both the monovalent ( $\text{H}_2\text{PO}_4^-$ ) & divalent ( $\text{HPO}_4^{2-}$ ) anions.
- Depending on the pH, the ratio of  $\text{H}_2\text{PO}_4^-$  to  $\text{HPO}_4^{2-}$  is 1:1 in acidosis, 1:4 at pH 7.4 and 1:9 in alkalosis.



# Regulatory mechanisms of phosphate homeostasis

- ❑ Phosphates absorbed from the intestine either actively through the cells or passively through the paracellular pathway.
- ❑ Once absorbed, inorganic phosphate enters into the extracellular compartment, particularly into the circulating plasma, where its concentration is tightly regulated by the three organs depicted here, parathyroid, bone, and kidney, interacting with one another through **three feedback loops**.



# Regulatory mechanisms of phosphate homeostasis

- ❑ Parathyroid glands produce parathyroid hormone (PTH), which, on the kidney, stimulates phosphate excretion and calcitriol synthesis; then, in turn, low phosphate and calcitriol directly inhibit PTH production.
- ❑ On the bone, PTH stimulates fibroblast growth factor 23 (FGF23) production and phosphate release following an increase in bone remodeling. FGF23 inhibits PTH secretion, but phosphate will tend to stimulate PTH production.
- ❑ FGF23, at the kidney level, stimulates urinary phosphate excretion and inhibits calcitriol production, tending to reduce serum phosphate levels by these two mechanisms.
- ❑ On the other hand, the renal PTH-stimulated calcitriol production stimulates FGF23 production by bone cells.
- ❑ These three counterregulatory loops maintain tightly controlled intestinal absorption and serum phosphate concentration.



# Hypophosphatemia

- ❖ **Hypophosphatemia** is defined when serum phosphate is below the lower limit of reference interval, around 2.5 mg/dL.
- ❖ The clinical manifestations of serum phosphate depletion depend on the length and degree of deficiency.
- ❖ Plasma concentrations less than 1.5 mg/dL may cause muscle weakness, acute respiratory failure, and decreased cardiac output.
- ❖ At very low serum phosphate ( $< 1$  mg/dL), tissue hypoxia may develop.





# Hypophosphatemia

- ❑ The three primary mechanisms leading to hypophosphatemia are:
  - I. transcellular shift of phosphorus (from extracellular volume to either soft tissues or bones).
  - II. poor dietary intake, especially when associated with impaired GI absorption or diarrhea.
  - III. increased phosphate excretion resulting from renal and nonrenal causes.

# Hyperphosphatemia

- ❑ The most common cause of hyperphosphatemia is the kidney's inability to excrete phosphate in CKD.
- ❑ In acute or chronic kidney failure, a decrease in the glomerular filtration rate (GFR) reduces the renal excretion of phosphate, resulting in hyperphosphatemia.
- ❑ Other causes include hypoparathyroidism, PTH resistance, or acromegaly.
- ❑ In renal failure due to decreased glomerular filtration, hyperphosphatemia and hyperparathyroidism can be present at the same time.
- ❑ Increased intake and a shift from the tissue to extracellular fluid, and acidosis are also causes of hyperphosphatemia.





# Measurement of Inorganic Phosphate

- ✓ The measurement of inorganic phosphate is based on its reactions with ammonium molybdate forming a phosphomolybdate complex at acidic condition.
- ✓ Most automated analyzers use a direct method to measure the absorbance of this complex at ultraviolet wavelength 340 nm that is directly proportional to the concentration of inorganic phosphate in the sample.
- ✓ The pH of the reaction has to be controlled to prevent spontaneous reduction of molybdate and solubilizing reagent needs to be used to prevent protein precipitation at low pH.





**Thank you for your  
attention**

FASTING BLOOD  
GLUCOSE

POST-PRANDIAL  
GLUCOSE

ORAL GLUCOSE  
TOLERANCE TEST

Lab. 2

Dr. Ghaidaa S. Al-Bana

- ❑ Continuous provision of energy is essential to maintain life.
- ❑ The most important energy substrates in mammals are glucose and fatty acids.
- ❑ After ingestion of food, their excess is stored to be released again in case of need.
- ❑ This storage-release pattern maintains the energy supply between meals, and in extreme circumstances can ensure an organism's survival for weeks and months.
- ❑ Continuous supply of glucose is essential for survival because in normal circumstances glucose is the only fuel which the brain can use.

## Introduction



❑ In spite of the fact that from time to time there is large demand for glucose, the amount of glucose present in the extracellular fluid is only about 20 g, the equivalent of 80 kcal. To maintain supply, glucose can be released into the circulation from the 'emergency store' of its polymer, glycogen.

❑ Glycogen is stored in the liver (approximately 75 g) and in muscle (400 g). This is equivalent to about 1900 kcal and can supply glucose for approximately 16 h of fasting.

## Introduction



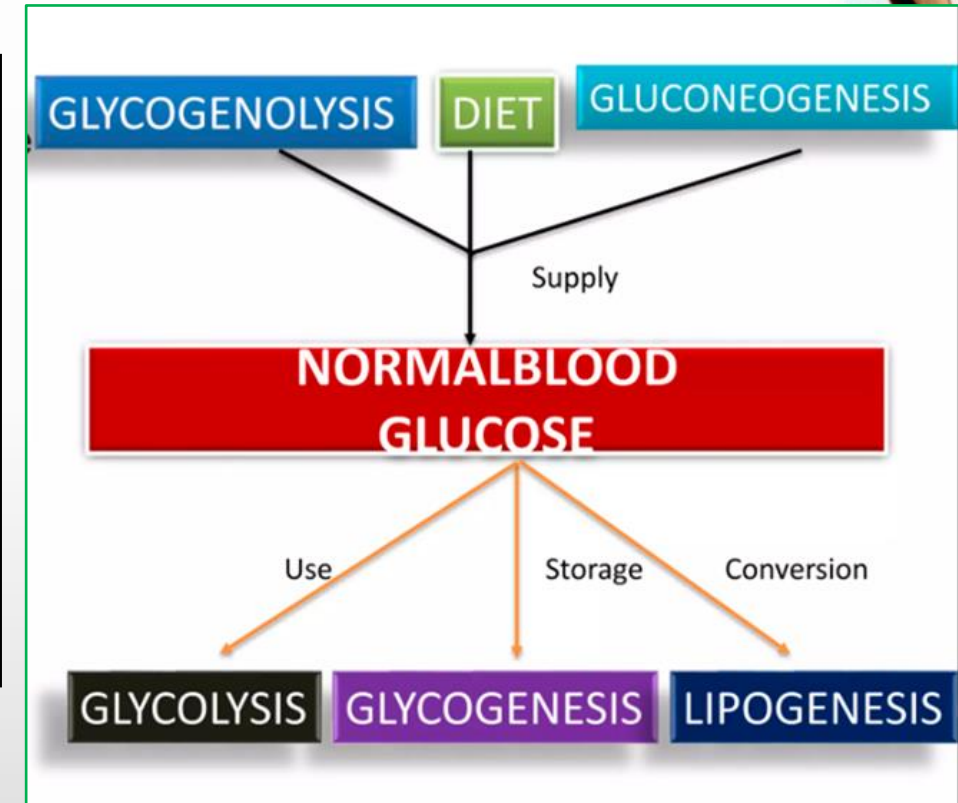
## Introduction

- ♪ When the period of fast is longer, another mechanism of glucose supply comes into play: its synthesis de novo from noncarbohydrate compounds known as **gluconeogenesis**.
- ♪ The body has virtually unlimited capacity for the accumulation of fat in the adipose tissue, and it is stored as esters of glycerol.
- ♪ Fatty acids support body energy needs during prolonged periods of fasting, and during prolonged exercise. In extreme circumstances, people can fast for as long as 60–90 days.



# Introduction

- Amino acids normally serve as substrates for synthesis of body's proteins.
- However, in certain situations they become energy substrates.
- During a prolonged fast or periods of metabolic stress induced by illness or injury, body proteins are degraded, and the released amino acids are converted into glucose in the course of **gluconeogenesis**.

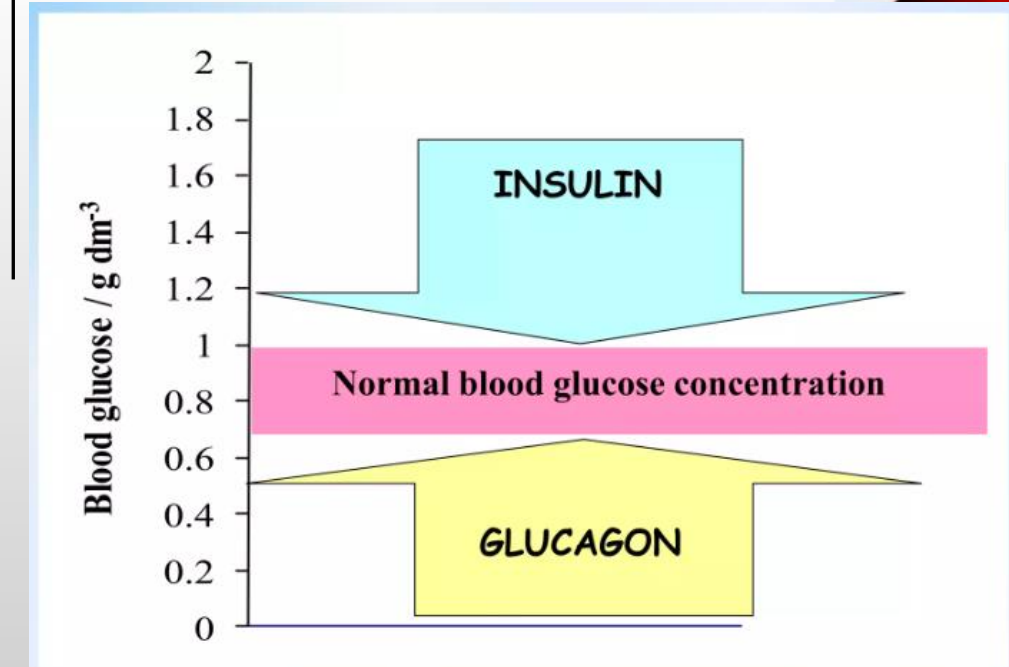
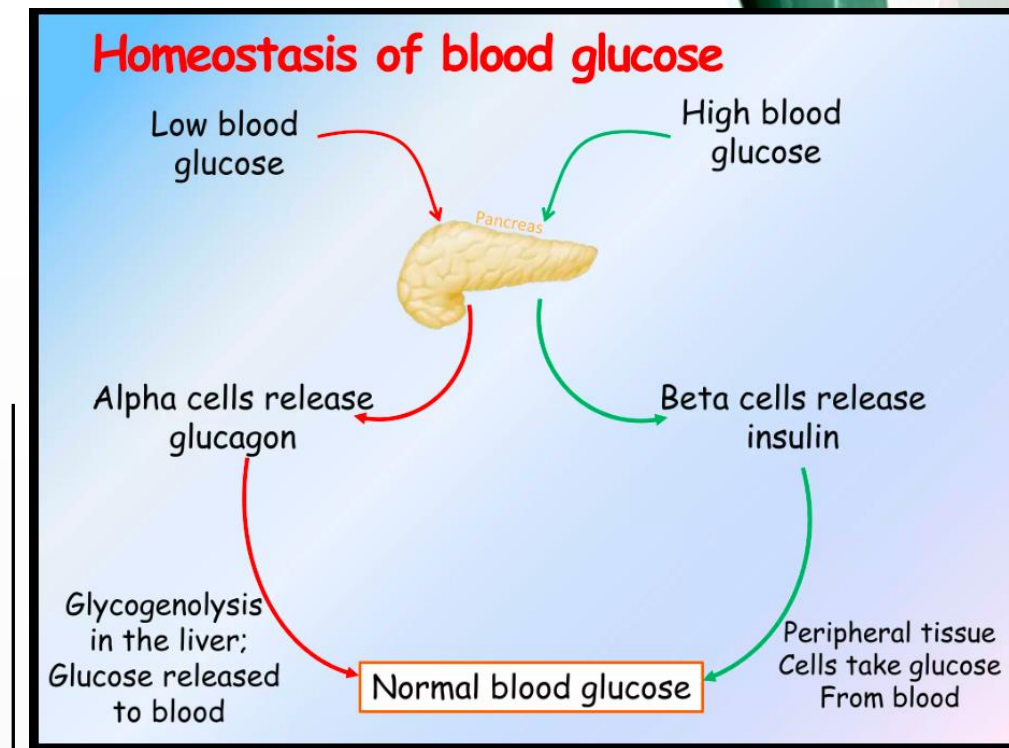


# Glucose Homeostasis

In order to ensure normal body function, the human body is dependent on a tight control of its blood glucose levels.

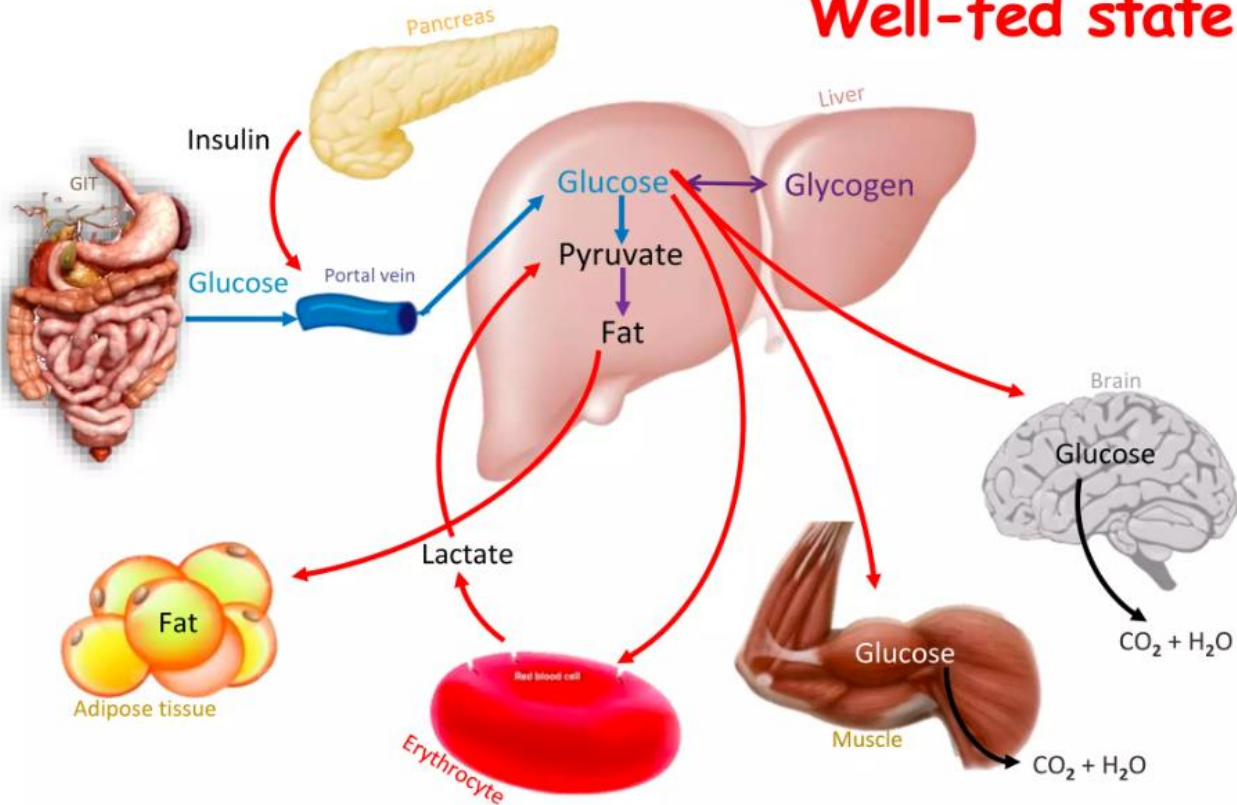
This is accomplished by a highly sophisticated network of various hormones and neuropeptides released mainly from the brain, pancreas, liver, intestine as well as adipose and muscle tissue.

Within this network, the pancreas represents a key player by secreting the blood sugar-lowering hormone insulin and its opponent glucagon.

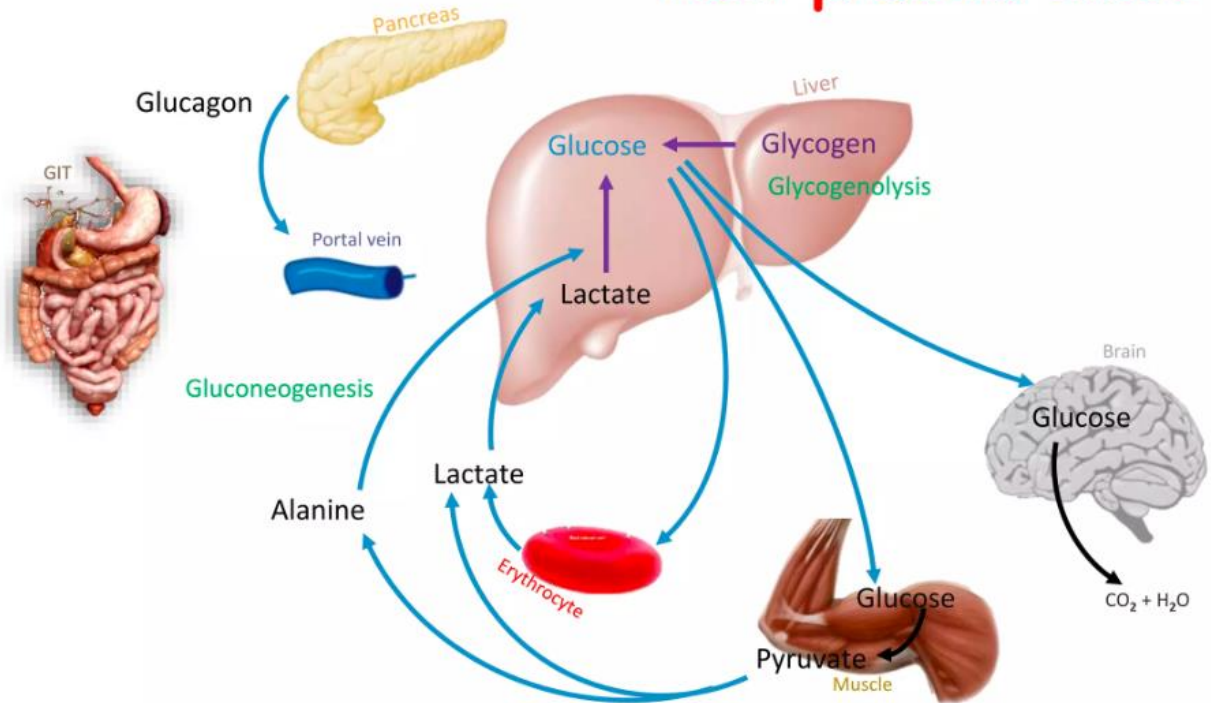


# Feed-Fast Cycle

## Blood glucose regulation during Well-fed state



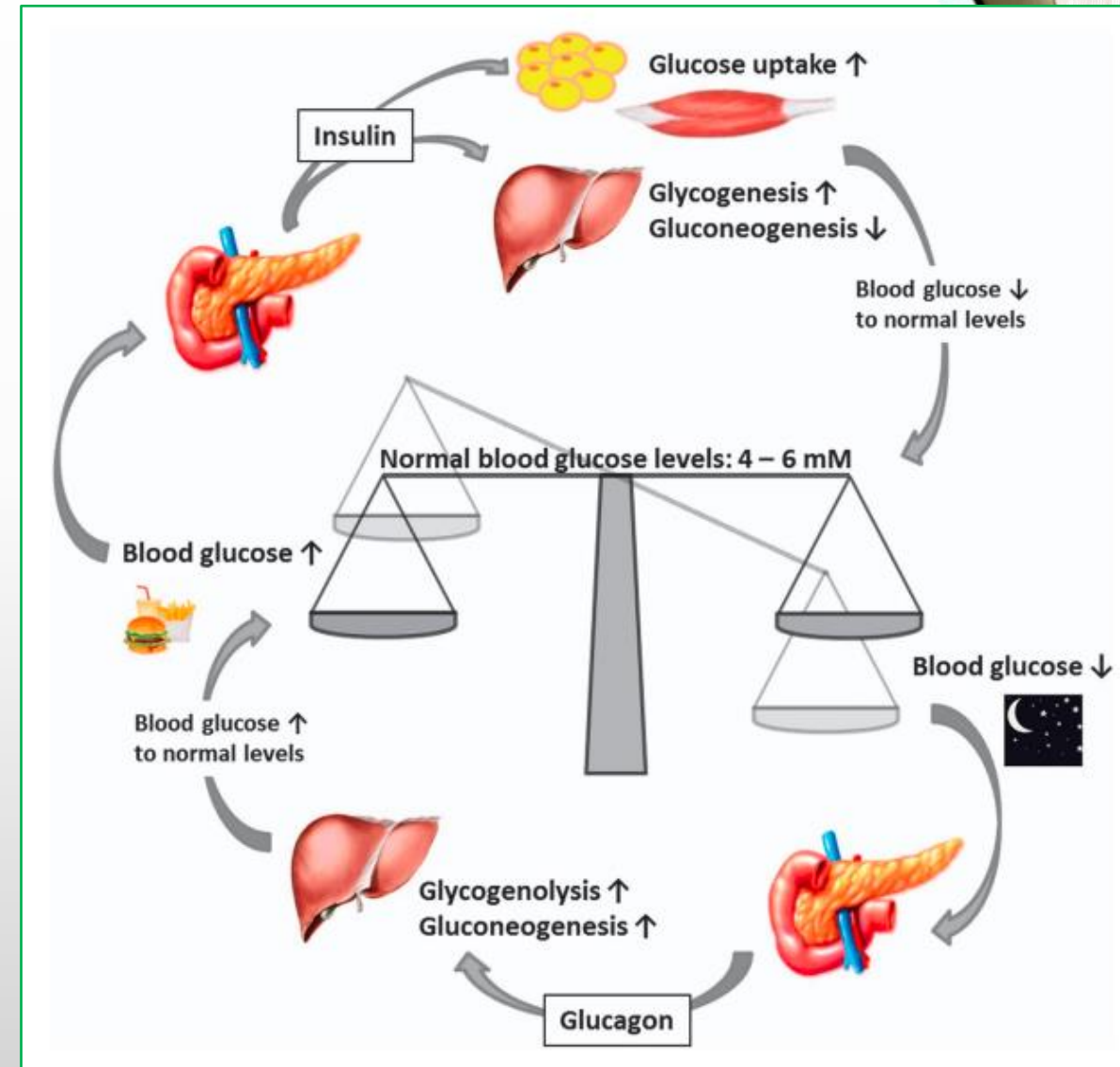
## Blood glucose regulation during Post-prandial state





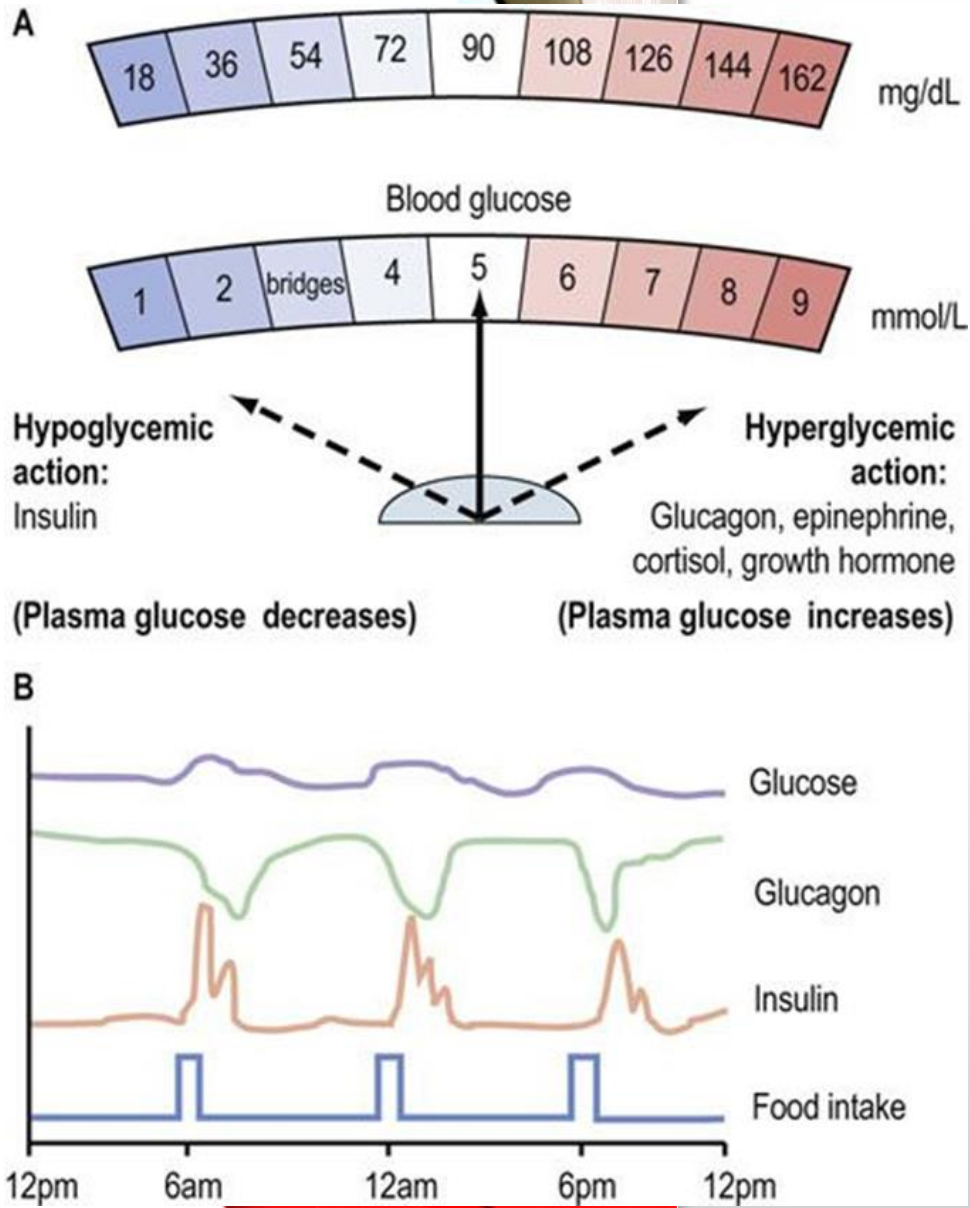
## ➤ Different organs and tissues handle fuels differently:

- ❑ At rest, **the brain** uses approximately 20% of all oxygen consumed by the body. Glucose is normally its only fuel.
- ❑ During starvation, however, the brain adapts to the use of ketone bodies as an alternative energy source.
- ❑ The two pathways that provide glucose are **glycogenolysis** and **gluconeogenesis**.
- ❑ When glucose concentration in the extracellular fluid decreases, it is first replaced by degrading liver glycogen.
- ❑ However, when the fasting period extends, gluconeogenesis is initiated.



- Gluconeogenesis takes place mostly in the **liver**, and the **kidneys** also contribute during prolonged fast. Its main substrates are **lactate** (from anaerobic glycolysis), **alanine** (from the amino acids released during breakdown of muscle protein) and **glycerol** (from the breakdown of triacylglycerols in the adipose tissue).
- Muscle uses both glucose and fatty acids as energy sources. During short-term exercise, glucose is the preferred substrate, however, at rest and during prolonged exercise, fatty acids are the main energy source.
- The myocyte cannot release glucose directly into the circulation because it does not contain the enzyme glucose-6-phosphatase. Thus, it uses glycogen only for its own energy needs.
- However, it does contribute to gluconeogenesis by releasing lactate, which is transported to the liver. No gluconeogenesis takes place in muscle.

## Hormonal Control Of Glucose Homeostasis

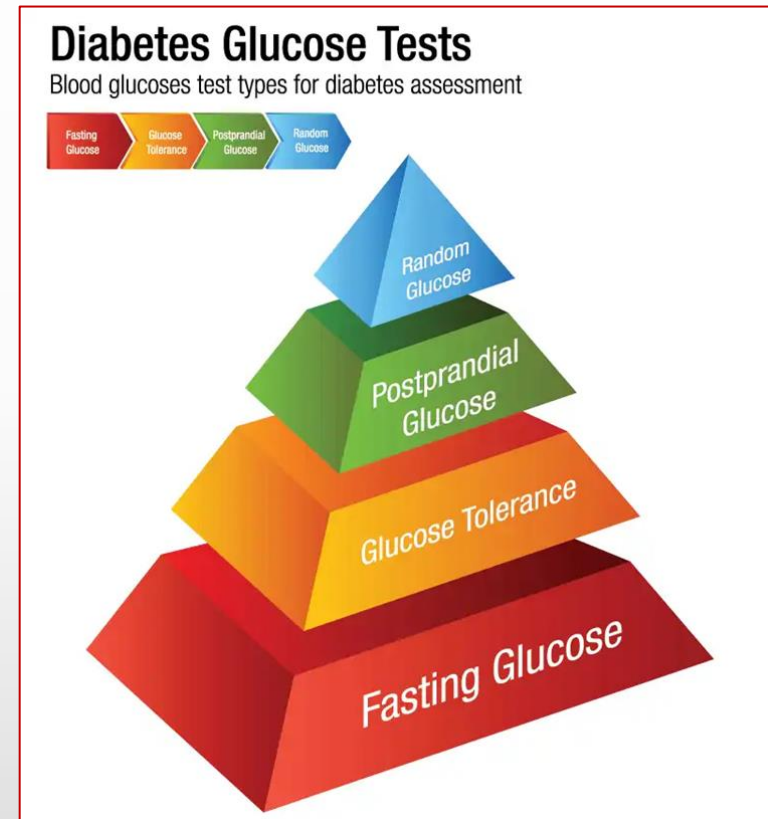


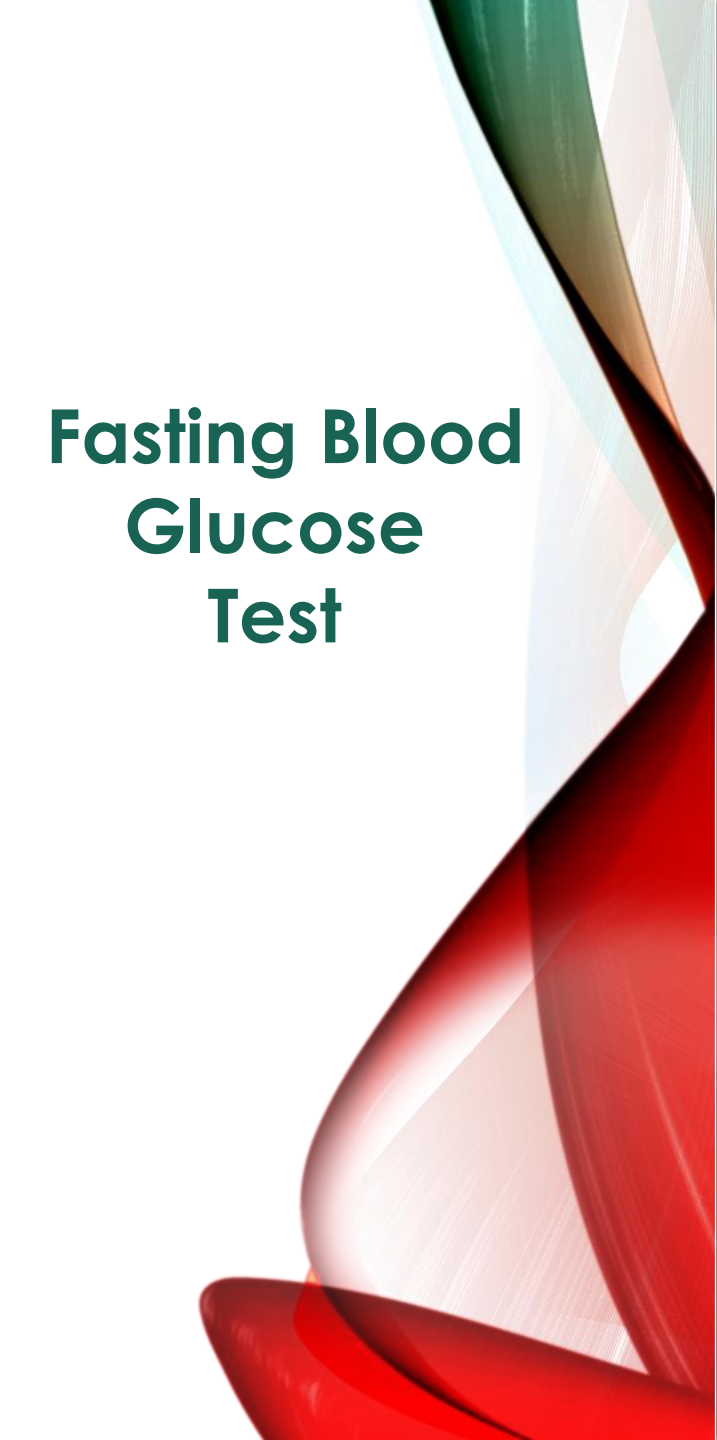
- ❖ (A) Plasma glucose concentration reflects the balance between the hypoglycemic (glucose-lowering) action of insulin and the hyperglycemic (glucose-increasing) action of the anti-insulin hormones.
- ❖ (B) Daily patterns of insulin and glucagon secretion, and corresponding plasma glucose concentrations. Plasma glucose concentration is maintained within a narrow range throughout the day.
- ❖ To obtain glucose concentrations in mg/dL, multiply the value in mmol/L by 18.



# Laboratory assessment of the plasma glucose concentration

- The measurement of plasma glucose concentration is the most important test of metabolism.
- It needs to be interpreted in relation to the feed–fast cycle.
- The best time to assess carbohydrate metabolism is after an 8–12 h fast when the glucose metabolism reaches steady state.
- Glucose concentration measured irrespective of the meal times is known as the **random plasma glucose**.
- It is useful for the diagnosis of hypoglycemia or severe hyperglycemia, but it is less helpful in assessing the significance of a mild hyperglycemia.



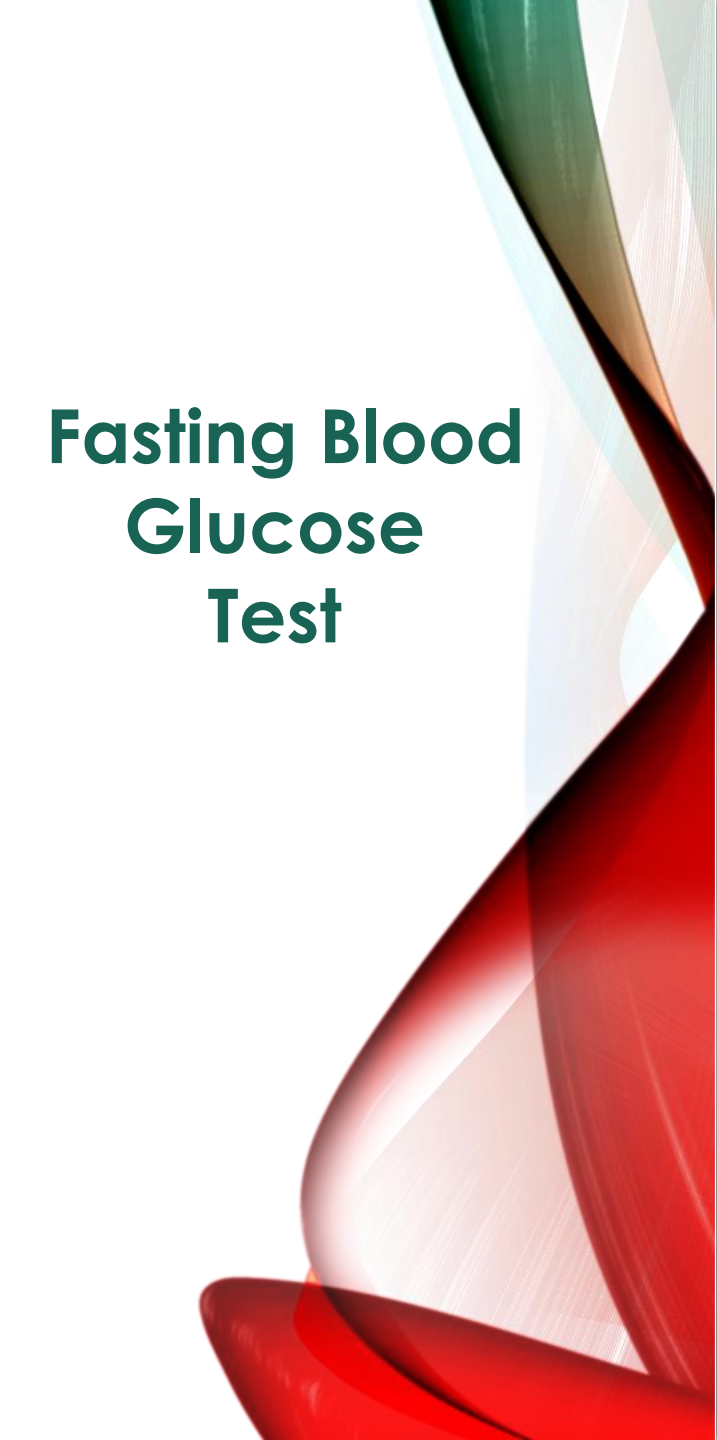


## Fasting Blood Glucose Test

♪ The measurements that are diagnostic for diabetes are the **fasting blood glucose concentration** (no caloric intake for about 10 h).

♪ Interpreting the glucose concentration, a clinician wants to know whether it is normal (normoglycemia), too high (hyperglycemia) or too low (hypoglycemia). Further interpretation of hyperglycemia includes the diagnosis of diabetes or identification of the intermediate (prediabetic) stages of metabolism.

♪ The fasting blood glucose in an individual is remarkably stable. The prediabetic abnormalities of carbohydrate metabolism are defined as **the impaired fasting glucose (IFG)** and **the impaired glucose tolerance (IGT)**. The American Diabetes Association (ADA) recommends the diagnosis of IFG. The World Health Organization (WHO) recommends diagnosis of IGT.

An abstract graphic on the left side of the slide shows a glass filled with a red liquid, possibly blood, with a green liquid being poured into it from the top. The glass is partially visible, and the liquids are swirling and mixing.

## Fasting Blood Glucose Test

### ♪ Indications:

According to the American Diabetes Association:

Screening to detect **pre-diabetes** (impaired fasting glucose [IFG] or impaired glucose tolerance [IGT]) and **diabetes** should be considered in:

1. Individuals >45 years of age, particularly in those with a body mass index (BMI) >25 kg/m<sup>2</sup>
2. Screening should also be considered for people who are <45 years of age and are overweight.
3. Individuals having additional risk factors for diabetes like strong family history.
4. Screening for diabetes in patients with hypertension or hyperlipidemia should be part of an integrated approach to reduce cardiovascular risk.

## Procedure:

- Explain to the patient the purpose of the test and the need for a blood sample to be drawn.
- Fasting of at least 8 hours is required prior to the test. Water is permitted.
- Insulin or oral hypoglycemic agents are to be withheld until after the blood sample is drawn.
- A 7-mL blood sample is drawn in a collection tube containing a glycolytic inhibitor such as sodium fluoride.

**Laboratory  
assessment of the  
plasma glucose  
concentration**



# Laboratory assessment of the plasma glucose concentration

- Normally, the fasting blood glucose concentration should remain below 6.1 mmol/L (110mg/dL).
- The IGT is characterized by a normal fasting level but an elevated concentration 2 h after glucose load.
- The IFG is defined as an '**intermediate**' **fasting plasma glucose** (higher than 6.0 mmol/L but lower than 7.0 mmol/L (126 mg/dL)).
- The fasting blood glucose of 7.0 mmol/L (126 mg/dL) or above, if confirmed, is diagnostic for diabetes.

Condition	Diagnostic criteria (mmol/L)	Diagnostic criteria (mg/dL)
Normal fasting plasma glucose	Below 6.1	Below 110
Impaired fasting glucose (IFG)	Equal or above 6.1 but below 7.0	Equal or above 110 but below 126
Impaired glucose tolerance (IGT)	Plasma glucose during OGTT, 2 h after 75 g load 7.8 or above, but below 11.1	Plasma glucose during OGTT, 2 h after 75 g load 140 or above, but below 200
<b>Diabetes mellitus*</b>		
Criterion 1	Random plasma glucose 11.1 or above†	Random plasma glucose 200 or above†
Criterion 2	Fasting plasma glucose 7.0 or above	Fasting plasma glucose 126 or above
Criterion 3	2 h value during OGTT 11.1 or above	2 h value during OGTT 200 or above
Criterion 4	HbA <sub>1c</sub> > 48 mmol/mol (6.5%)	

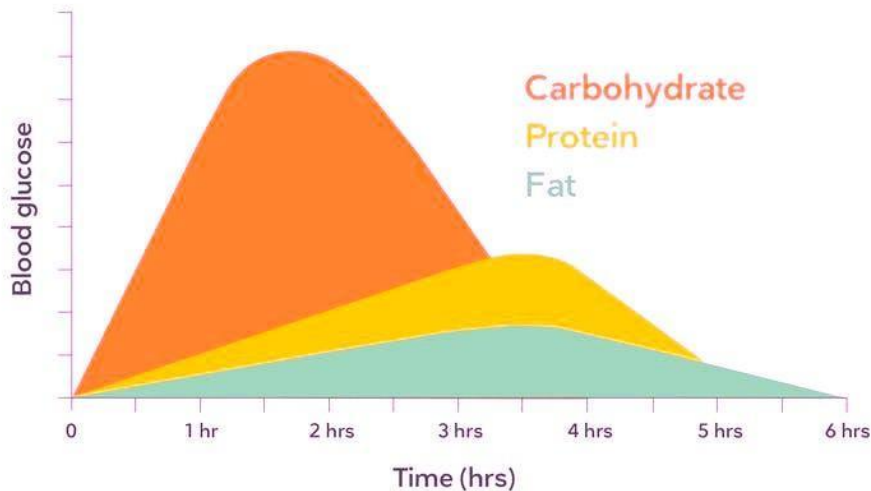
## Post-Prandial Blood Glucose Test

🎵 Post Prandial glucose test or PPGT test measures blood sugar levels after 2 hours of eating. Usually, blood glucose levels increase and reach the peak of 1h after eating and then return to premeal levels within 2 to 3 hours. This variance in plasma glucose is controlled by the insulin response to food intake

🎵 In non-diabetic patients, 2h post- prandial blood glucose levels are usually  $<140$  mg/dL (7.8 mmol/l).

🎵 In patients with type 2 diabetes, the insulin response is decreased or absent, resulting in elevated post-prandial glucose.

Effects of nutrients on blood glucose over time







## Oral Glucose Tolerance Test (OGTT)

- ♪ OGTT assesses blood glucose response to a carbohydrate load.
- ♪ WHO recommends that the OGTT is performed on all individuals whose fasting plasma glucose falls into the IFG category.
- ♪ OGTT must be performed under standard conditions:
  - The patient should attend in the morning, after an approximately 10 h fast.
  - The test should not be performed during, or immediately after, an acute illness.
  - During the test, the fasting plasma glucose is measured first.
  - The patient is then given a standard quantity of glucose to drink (75 g in 300 mL of water) and the plasma glucose concentration is measured again after 120 min.
  - In some protocols glucose is measured after 20, 60 and 120 min.

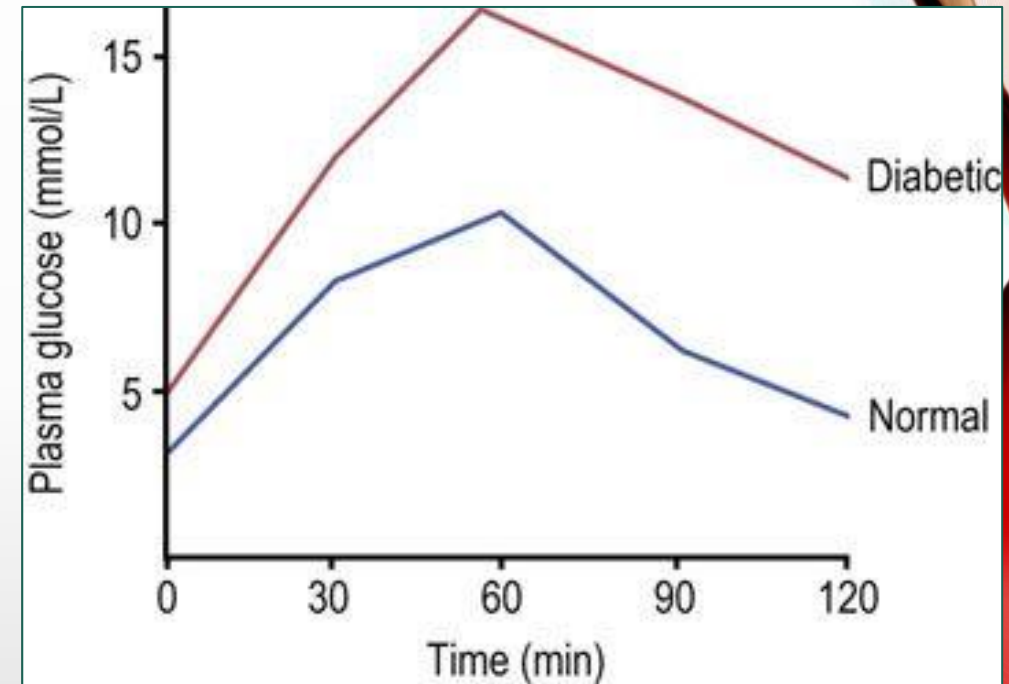
# Oral Glucose Tolerance Test (OGTT)

## Indications

1. In patient with transient or sustained glycosuria, who have no clinical symptoms of diabetes with normal FBS & PPBG.
2. to confirm a diagnosis of diabetes or prediabetes in individuals who have borderline results on other glucose tests.
3. In patient with symptoms of diabetes but with no glycosuria and normal fasting blood glucose.
4. Used to diagnose gestational diabetes, excessive weight gaining is noticed during pregnancy, with a past history of big baby ( $> 4$  kg ).
5. In persons with strong family history of diabetes but no symptoms.

# Oral Glucose Tolerance Test (OGTT)

- Normally, plasma glucose should reach peak concentration after approximately 60 min and should return to a near-fasting state within 120 min.
- If it remains above 11.1 mmol/L (200 mg/dL/min) in the 120 min sample, diabetes is diagnosed, even if the fasting blood glucose were normal.
- Nondiabetic fasting blood glucose with the post-load concentration between 6.1 and 7.8 mmol/L (100–140 mg/dL) signifies IGT.



FASTING FBS	RANDOM RBS	POST-PRANDIAL PPBS
<ul style="list-style-type: none"><li>• 70-110mg/dL</li><li>• 4-6.1mmol/L</li></ul>	<ul style="list-style-type: none"><li>• &lt; 140 mg/dl</li><li>• &lt;7.8 mmol/l</li></ul>	<ul style="list-style-type: none"><li>• 140 mg/dL</li><li>• 7.8 mmol/L</li></ul>

## Urine glucose is not a diagnostic test for diabetes

- At a normal plasma concentration, glucose filtered through the renal glomeruli is reabsorbed in the proximal kidney tubules, and none appears in the urine.
- The urinary threshold for glucose reabsorption is approximately 10.0 mmol/L (180 mg/dL).
- At higher concentrations, the reabsorptive capacity of the renal tubular transport system is exceeded, and glucose appears in the urine (this is known as **glucosuria**).
- Note that a healthy person may have a low renal glucose threshold and thus show glucosuria at nondiabetic blood glucose levels.
- Therefore, diabetes cannot be diagnosed on the basis of urine testing alone.

# Urinary albumin excretion is important in the assessment of diabetic nephropathy

- The development of diabetic nephropathy can be predicted by detecting minute amounts of albumin in urine (**microalbuminuria**).
- To do this, laboratories employ a method that is more sensitive than the conventional one used for the measurement of serum albumin.
- The test is positive if more than 200 mg of albumin is excreted in urine over 24 h. Urine protein above 300 mg/day signifies evident proteinuria.



**THANK YOU FOR  
YOUR TIME**

