

**Al-Furat Al-Awsat Technical University**

**College of Medical Rehabilitation and Prosthetics**

**Medical Rehabilitation Department and Prosthetics**



Al-Furat Al-Awsat Technical University

**\*\*College of Medical Rehabilitation and Prosthetics\*\***

Assistant Lecturer Shaimaa Al-Hammami

**2025/2026**

**Lecture 1**

**Introduction to Biochemistry**

## **Introduction to Biochemistry**

Biochemistry is the branch of science that studies the chemical processes within living organisms. It bridges biology and chemistry, helping us understand how molecules interact to sustain life.

Think of biochemistry as the language of life: proteins, lipids, carbohydrates, and nucleic acids are the words, and their interactions form the sentences that describe cellular function. Without biochemistry, we cannot explain how cells generate energy, replicate, or respond to their surroundings.

### **\* Cell: Biochemical Aspects**

The cell is the basic structural and functional unit of life. It is the smallest unit capable of carrying out all vital biological processes.

- Function / Importance:
  1. metabolism
  2. energy production
  3. growth and division
  4. response to stimuli
  5. maintaining homeostasis

### **\*Cell Components – Definitions & Functions**

#### **1-Cell Membrane**

**Definition:** A thin, flexible membrane that surrounds the cell.

**Function:** Controls the movement of substances into and out of the cell and protects the cell.

#### **2- Cytoplasm**

**Definition:** A jelly-like substance that fills the cell and contains the organelles.

**Function:** Site of many metabolic reactions and supports cell structures.

#### **3- Nucleus**

**Definition:** A membrane-bound organelle that contains the cell's DNA.

**Function:** Controls cell activities and stores genetic information.

#### **4- Mitochondria**

**Definition:** Double-membrane organelles known as the powerhouse of the cell.

**Function:** Produce ATP (energy) through cellular respiration.

#### **5-Ribosomes**

**Definition:** Small particles made of RNA and proteins found in the cytoplasm or on the rough ER.

**Function:** Responsible for protein synthesis.

#### **6- Endoplasmic Reticulum (ER)(Rough ER**

**Definition:** A network of membranes covered with ribosomes.

Function: Protein synthesis and transport.

### 7- Smooth ER

Definition: A membrane network without ribosomes.

Function: Lipid synthesis, detoxification, and calcium storage.

### 8- Golgi Apparatus

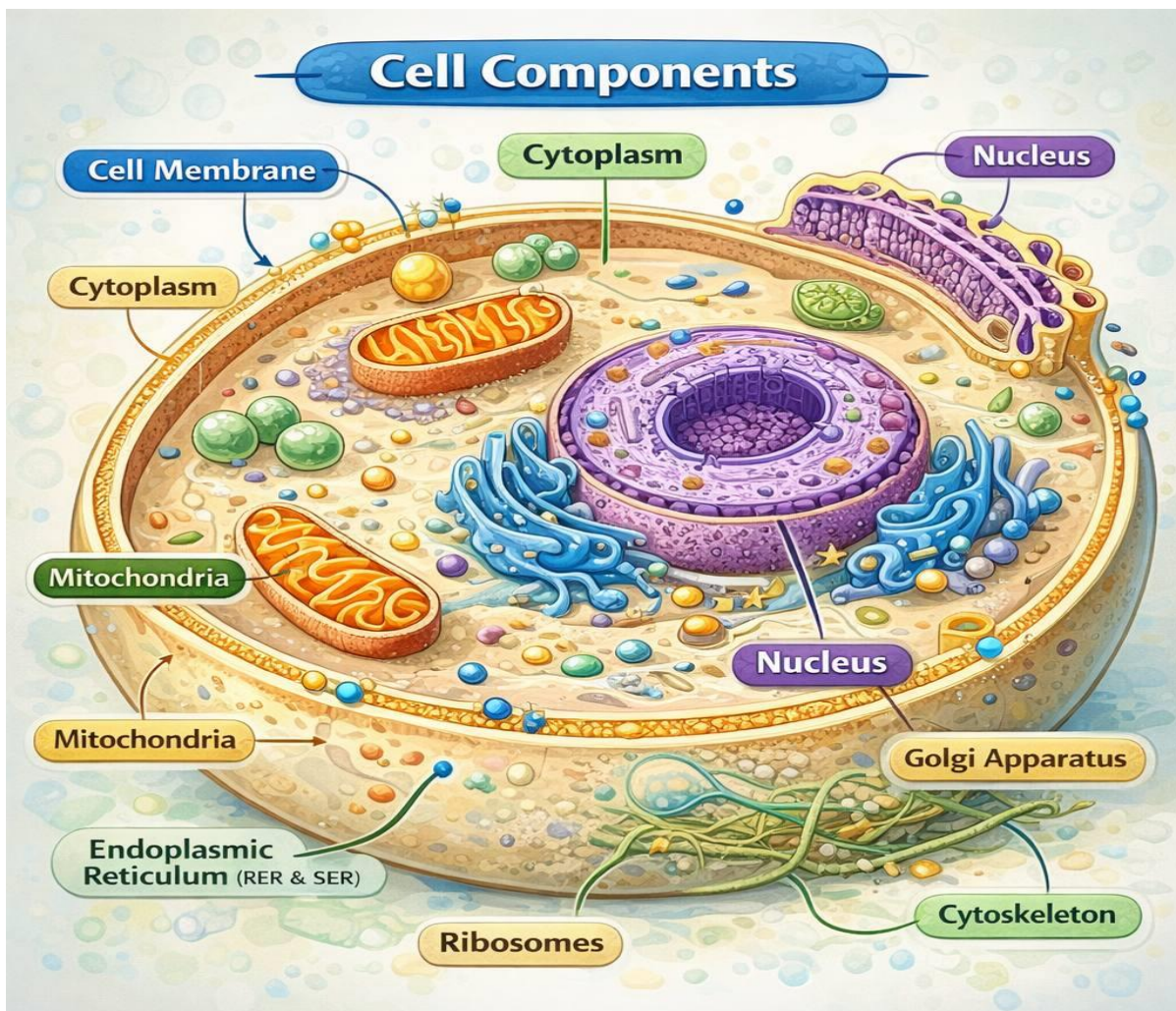
Definition: A stack of flattened membrane sacs.

Function: Modifies, packages, and transports proteins and lipids.

### 9- Lysosomes

Definition: Membrane-bound organelles containing digestive enzymes.

Function: Break down waste materials and damaged cell parts.



### \* Cell Membrane Structure

**Definition:** The cell membrane is a thin, flexible boundary that surrounds the cell and separates the internal environment from the external environment.

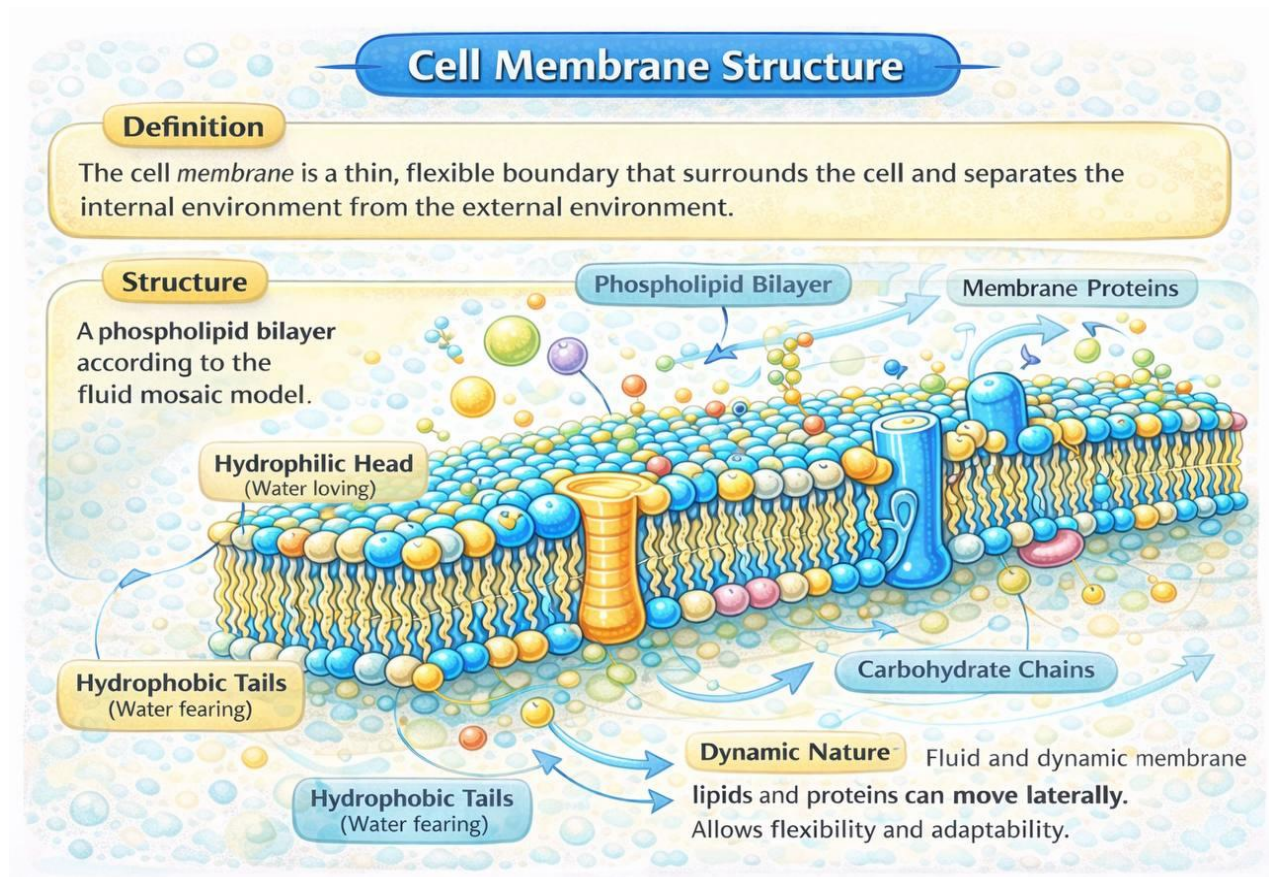
**Structure:** The cell membrane is composed of a phospholipid bilayer according to the fluid mosaic model.

**Each phospholipid molecule has:**

- **Hydrophilic head** (water-loving) that faces the aqueous environment inside and outside the cell.
- **Hydrophobic tails** (water-repelling) that face inward, away from water.

Embedded within this lipid bilayer are membrane proteins, cholesterol, and carbohydrate chains, which help in transport, communication, and structural support.

**Dynamic Nature:** The membrane is fluid and dynamic, meaning lipids and proteins can move laterally within the membrane, allowing flexibility and adaptability.



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**2025/2026**

**Lecture 2**

**Diabetes Mellitus**

## Introduction

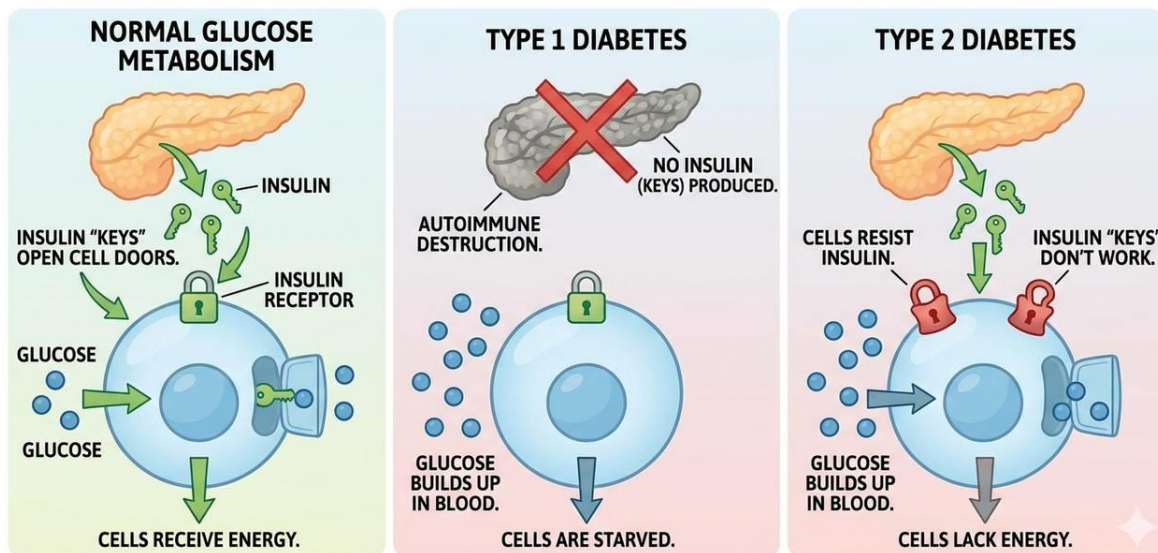
Why is Diabetes Important for You?

As future specialists in Physical Therapy and Prosthetics & Orthotics, you will deal with diabetic patients daily. Diabetes is the leading cause of non-traumatic lower-limb amputations and physical disabilities.

### \Clinical Classifications (Review)

- Type 1 (T1DM): Total insulin deficiency.
- Type 2 (T2DM): Insulin resistance.
- Gestational: Occurs during pregnancy.

## UNDERSTANDING INSULIN ACTION & DIABETES TYPES

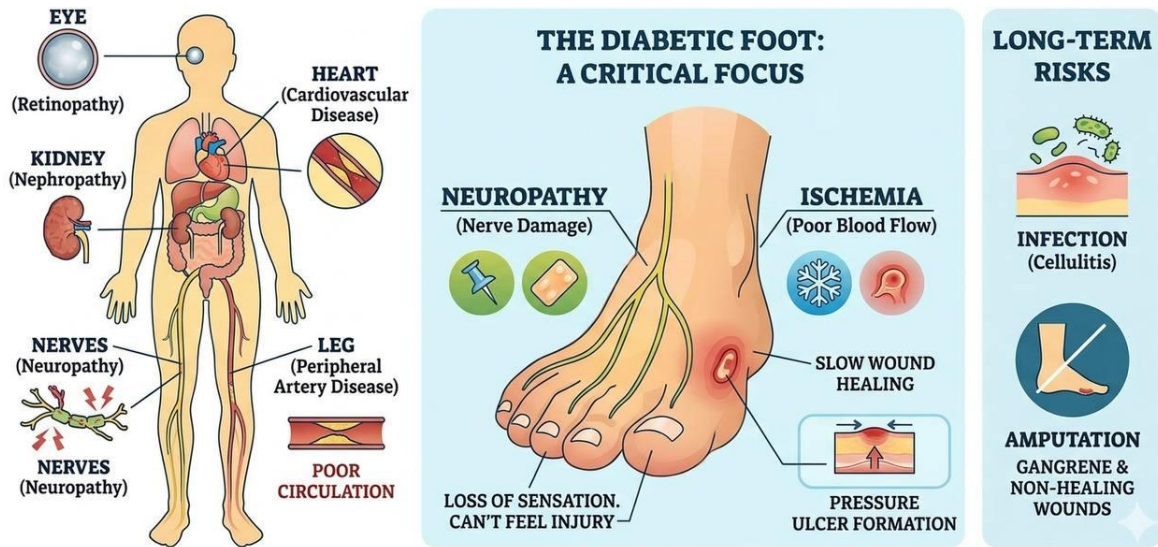


## Focus on "The Diabetic Foot" (Practical Aspect)

This is the most critical area for Prosthetics & Orthotics students.

- Diabetic Neuropathy:** Loss of sensation. Patients can't feel pain, heat, or pressure.
- Peripheral Artery Disease (PAD):** Poor blood flow to the legs, leading to slow healing.
- Foot Ulcers:** Small wounds that turn into deep infections.

## CHRONIC COMPLICATIONS OF DIABETES MELLITUS



## Rehabilitation & Prosthetic Challenges

When working with a diabetic patient, you must check for:

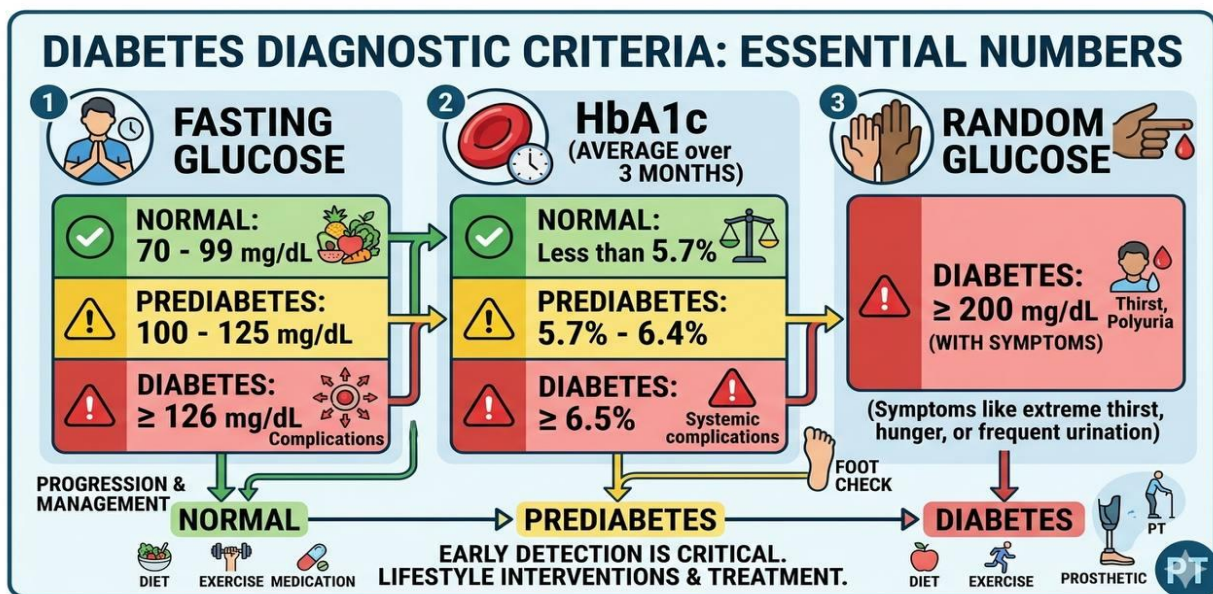
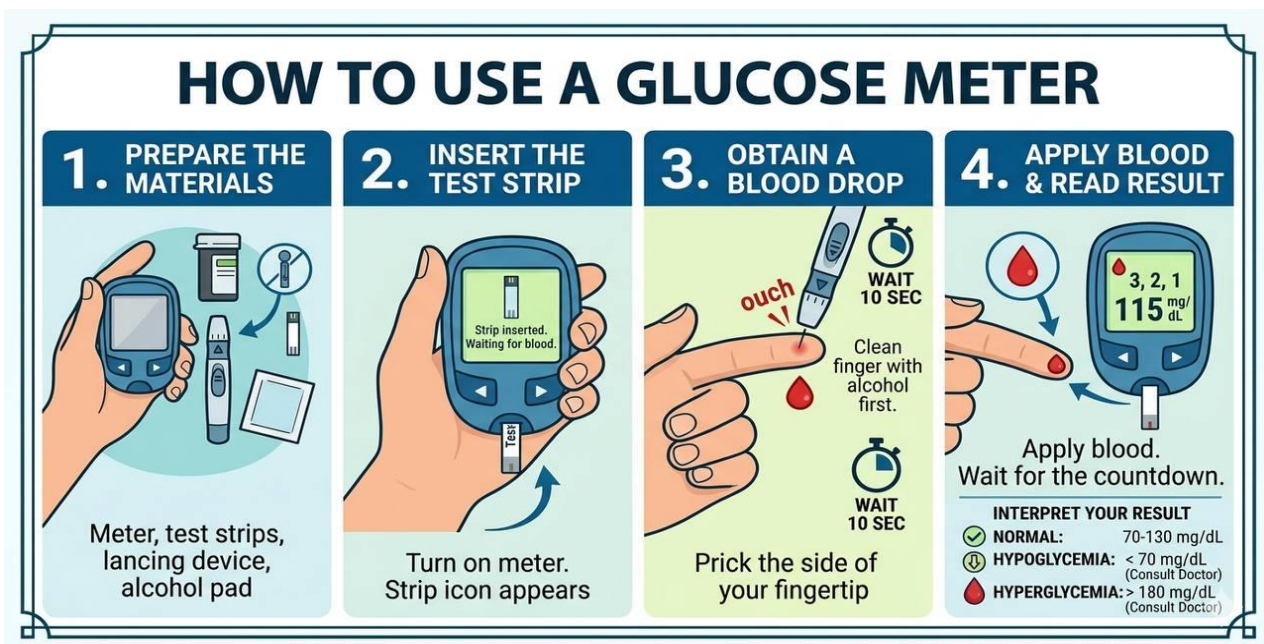
- 1- Skin Integrity: Inspect the stump/limb for redness or blisters caused by the prosthetic socket.
- 2- Sensory Testing: Perform a Monofilament Test (checking for sensation loss.)
- 3- Wound Care: Ensure the prosthetic device does not cause pressure on existing ulcers.
- 4- Fluctuating Limb Size: Diabetic patients often have edema (swelling), which changes how a prosthetic fits throughout the day.
- 5- Emergency Situations in the Rehab Clinic

You must recognize these two during exercise or fitting sessions:

• **Hypoglycemia** (Low Sugar): Trembling, sweating, confusion. (Give sugar/juice immediately.)

• **Hyperglycemia** (High Sugar): Extreme thirst, fruity breath, fatigue

## 6. Laboratory Diagnosis & Monitoring



## 7- Summary for First-Year Students

- Goal: Prevention of amputation through proper footwear (Orthotics.)
- Role: Educating the patient on daily foot inspections.
- Fit: Prosthetics must be custom-fitted to avoid "pressure points."

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**Lecture 3**

**Clinical Applications of  
Kidney Function Tests  
(KFT)**

# Introduction

**The Kidney:** is a bean-shaped organ that acts as a natural filter for the body. Its primary function is to remove waste products and excess water from the blood to produce Urine.

## Function

- 1- Filtration: Clears toxins and waste (like Urea and Creatinine.)
- 2- Fluid Balance: Regulates the amount of water and salt in the body.
- 3- Hormone Production:
  - Erythropoietin: For red blood cell production.
  - Renin: For blood pressure control.
  - Vitamin D Activation: Essential for Bone Strength (Critical for Prosthetics/P&O).

## Core Blood Chemistry Tests

### A. Serum Creatinine (SCr)

- Definition: A waste product from muscle metabolism (creatine breakdown)
- Reference Range: 0.7 - 1.3 mg/dL (Men) | 0.6 - 1.1 mg/dL (Women)
- P&O Insight: Amputees often have lower muscle mass. Therefore, a "normal" creatinine level might actually hide early kidney dysfunction because they produce less creatinine.

### B. Blood Urea Nitrogen (BUN)

- Definition: Measures nitrogen in the blood that comes from the waste product urea (protein breakdown)
- Reference Range: 7 - 20 mg/dL.
- Clinical Note: High BUN (Azotemia) causes fatigue and mental confusion, which affects a patient's ability to follow complex rehabilitation instructions.

## C. Estimated Glomerular Filtration Rate (eGFR)

**Definition:** GFR is the best measure of kidney function. It is the volume of fluid (blood) filtered by the kidneys' tiny filters (glomeruli) every minute.

Normal Range:

- Normal: > 90 mL/min.
- Kidney Failure: < 15 mL/min.

## Urinalysis: The "Liquid Biopsy"

• **Proteinuria (Albumin):** Presence of protein in urine. In rehab, this often indicates systemic vascular disease (like Diabetes), which also causes Peripheral Neuropathy (loss of sensation in limbs).

• **Specific Gravity:** Measures urine concentration. Low values suggest the kidney cannot conserve water, leading to dehydration and orthostatic hypotension (dizziness when standing up for gait training.)

### CLINICAL APPLICATIONS OF KIDNEY FUNCTION TESTS IN MEDICAL REHABILITATION AND PROSTHETICS.

**1 THE REHABILITATION NEED.**

1. STUMP VOLUME & EDEMA CONTROL

2. BONE STRENGTH (Renal Osteodystrophy Risk)

3. MUSCLE & NERVE FUNCTION (Electrolytes)

**2 CORE BLOOD & URINE TESTS.**

BLOOD → SERUM CREATININE (SCr)

BLOOD → BUN (Blood Urea Nitrogen)

URINE → URINALYSIS

UREA CYCLE

**3 CLINICAL CORRELATION FOR P&O STUDENTS.**

| TEST RESULT                | IMPACT ON REHAB                    | ACTION REQUIRED                          |
|----------------------------|------------------------------------|--|
| HIGH CREATININE / LOW eGFR | Risk of Bone Fractures, Fatigue    | Modify Exercise Intensity, Monitor BP    |
| PROTEINURIA                | Peripheral Neuropathy, Skin Ulcers | Daily Skin Inspections of Residual Limb  |
| EDEMA / FLUID RETENTION    | Stump Volume Fluctuates            | Use Shrinker Socks, Delay Socket Casting |

REHABILITATION MEDICINE LECTURE SERIES

## Practical part

| UREA<br>Measurement (Kinetic Enzymatic Method)  |   | CREATININE<br>Measurement (Kinetic Jaffe Method)   |   |
|---|---|--|---|
| <b>1 SAMPLE COLLECTION</b><br><br>Centrifuge Blood to get Serum   | <b>2 REAGENT PREPARATION</b><br><br>Mix R1 + R2 (Working Reagent) | <b>1 SAMPLE COLLECTION</b><br><br>Centrifuge Blood to get Serum  | <b>2 REAGENT PREPARATION</b><br><br>Ready-to-use Reagents |
| <b>3</b><br><p>Urea + Urease → Ammonia</p> <p>Ammonia + α-Ketoglutarate + NADH (GLDH) → Glutamate + NAD<sup>+</sup></p> <p>Color decrease proportional to Urea.</p> |   | <b>3</b><br><p>Creatinine + Alkaline Picrate → Red Complex</p> <p>Red-Orange Complex</p> <p>Color increase proportional to Creatinine.</p>           |   |
| <b>4 CALCULATION</b><br><br>$\text{Urea (mg/dL)} = \frac{\Delta A(\text{Sample})}{\Delta A(\text{Calibrator})} \times \text{Calibrator Conc.}$                      |   | <b>4 CALCULATION</b><br><br>$\text{Creatinine (mg/dL)} = \frac{\Delta A(\text{Sample})}{\Delta A(\text{Calibrator})} \times \text{Calibrator Conc.}$ |   |
| <b>KINETIC MEASUREMENT AT 340 nm (Urea)   510 nm (Creatinine) USING AUTOMATED OR SEMI-AUTOMATED ANALYZER</b>  |   |  |   |

## Scientific References & Sources

- 1- National Kidney Foundation (NKF): KDOQI Clinical Practice Guidelines (<https://www.kidney.org/professionals/guidelines>) - The primary source for eGFR staging.
- 2-Mayo Clinic: Kidney Function Tests Overview (
- 3- Journal of Rehabilitation Research & Development (JRRD): Impact of Comorbidities on Amputation Rehab
4. Lab Tests Online (AACC): Understanding Urinalysis and BUN

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**Lecture 4**

**Clinical Applications of**

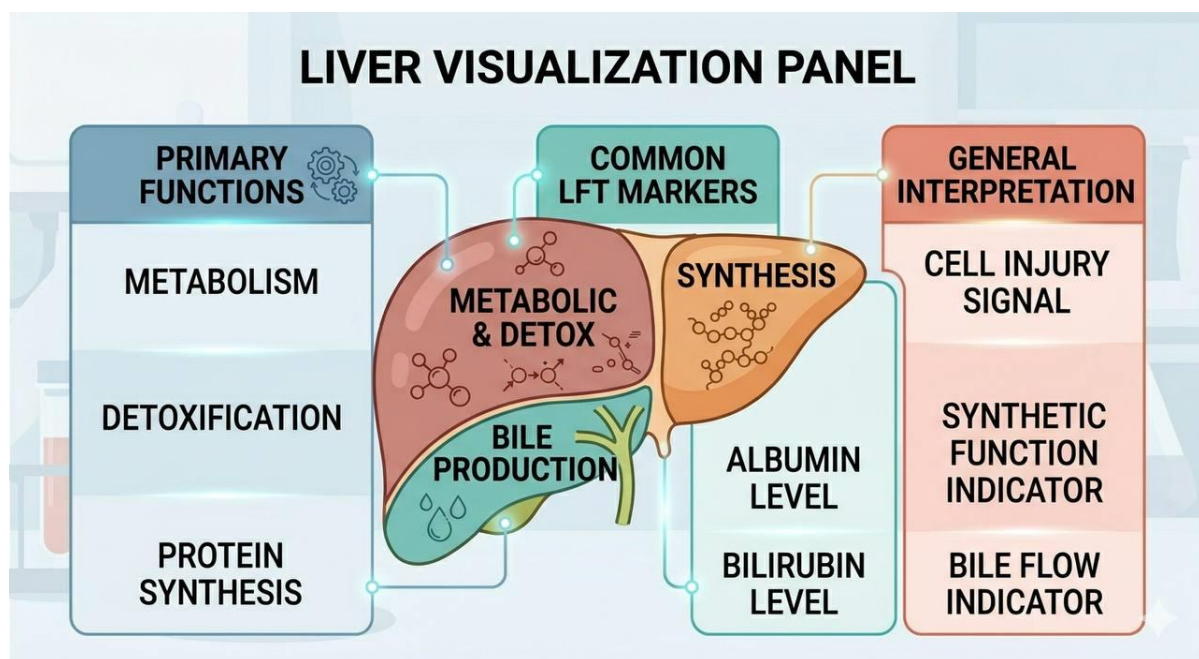
**Liver Function Tests (LFTs)**

## Introduction

**The liver** is the largest internal organ and the primary metabolic hub of the human body. For rehabilitation specialists, understanding liver health is vital because hepatic dysfunction can lead to muscle wasting, neurological issues (encephalopathy), and poor wound healing in prosthetic users.

Key Functions of the Liver:

- Metabolism: Processes nutrients from food and converts them into energy.
- Detoxification: Filters toxins, drugs, and metabolic waste from the blood.
- Synthesis: Produces essential proteins like Albumin (maintains fluid balance) and Clotting Factors (prevents bleeding.)
- Bile Production: Secretes bile to aid in the digestion and absorption of fats.



## Major Liver Function Tests (LFTs)

LFTs are a group of blood tests used to measure specific enzymes and proteins. They are generally categorized into two types: Markers of Liver Injury and Markers of Synthetic Function.

## A. Markers of Liver Injury (Enzymes)

### 1- ALT (Alanine Aminotransferase):

- Found mainly in the liver.
- Clinical Significance: High levels indicate acute liver damage (e.g., hepatitis.)

### 2- AST (Aspartate Aminotransferase):

- Found in liver, heart, and skeletal muscles.
- Rehab Relevance: High AST with normal ALT might suggest muscle injury rather than liver disease.

### 3- ALP (Alkaline Phosphatase):

- Related to bile ducts and bone turnover.
- Rehab Relevance: Elevated in bone healing or bone diseases.

### 4- GGT (Gamma-Glutamyl Transferase):

- Specific for bile duct injury and alcohol consumption.

## B. Markers of Synthetic Function

### 1- **Albumin:** The main protein made by the liver.

- Rehab Relevance: Low levels cause Edema (swelling), which significantly affects the fit of prosthetic sockets and orthotic braces.

### 2- **Bilirubin:** A waste product from the breakdown of red blood cells.






- Clinical Sign: High levels cause Jaundice (yellowing of skin/eyes)

### 3- PT/INR (Prothrombin Time)

- Measures how long it takes blood to clot.

## Reference Ranges (Standard)

**NORMAL REFERENCE RANGES: LIVER FUNCTION TESTS (LFTs)**

|   |                      |
|---|----------------------|
| <b>ALT (Alanine Aminotransferase)</b>    | <b>7–55 U/L</b>      |
| <b>AST (Aspartate Aminotransferase)</b>  | <b>8–48 U/L</b>      |
| <b>ALP (Alkaline Phosphatase)</b>        | <b>40–129 U/L</b>    |
| <b>Albumin</b>                           | <b>3.5–5.0 g/dL</b>  |
| <b>Total Bilirubin</b>                   | <b>0.1–1.2 mg/dL</b> |

## Practical Part: Laboratory Procedure



## References:

- Wallach's Interpretation of Diagnostic Tests.
- Guyton and Hall Textbook of Medical Physiology.
- Mayo Clinic: Liver Function Tests Overview.

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**Lecture 5**

**(Lipids Profile)**

## Introduction

**The Lipid Profile** is a panel of blood tests used to evaluate the status of lipid metabolism and cardiovascular risk. Lipids are essential fats, but abnormal levels are strongly linked to heart disease and peripheral vascular disease.

A standard profile, as recommended by the National Cholesterol Education Program (NCEP), measures four primary components:

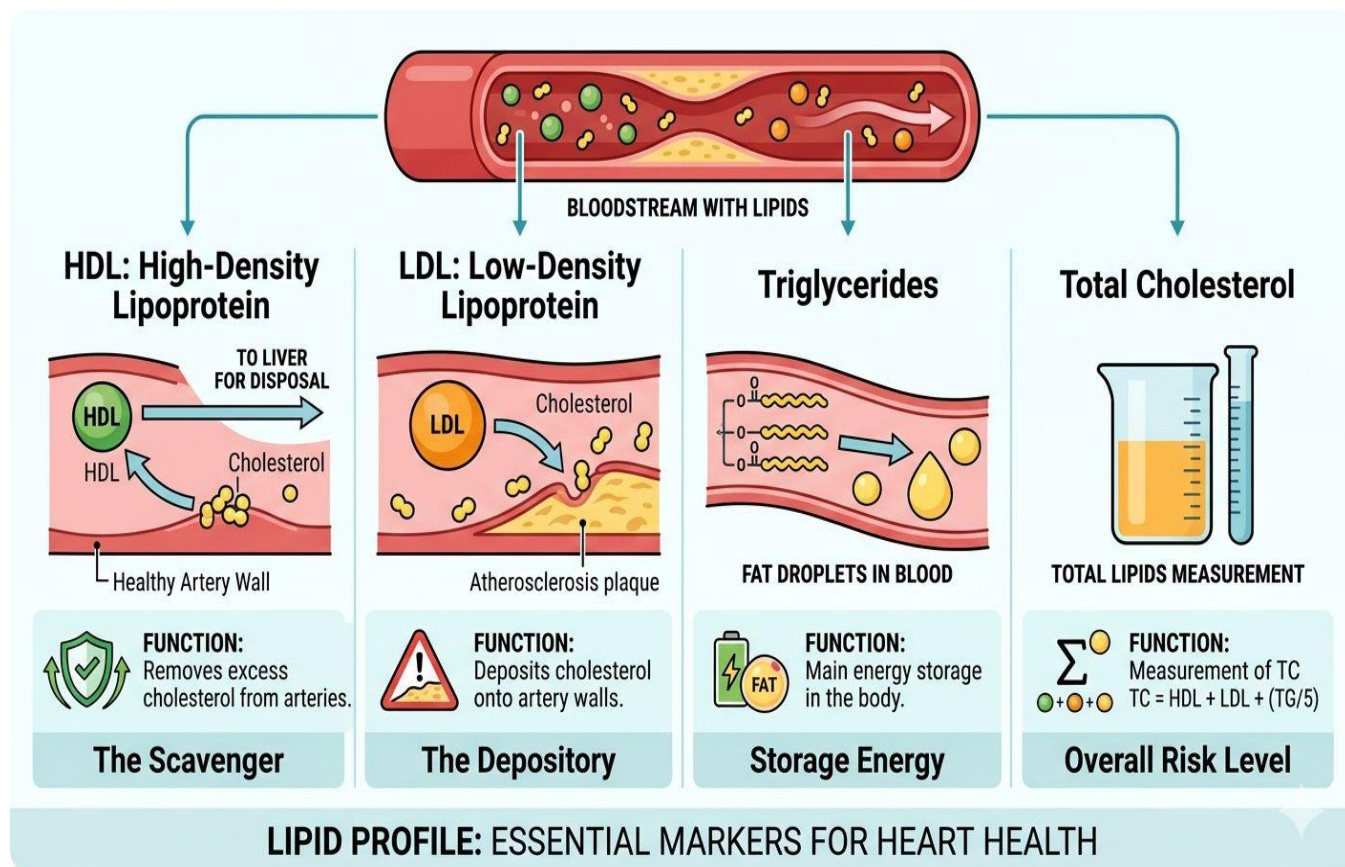
**1- Total Cholesterol (TC):** The entire amount of cholesterol in all lipoprotein fractions.

**2- High-Density Lipoprotein Cholesterol (HDL-C):** Known as "Good Cholesterol," it helps remove excess cholesterol from the arteries and transports it to the liver.

**3- Low-Density Lipoprotein Cholesterol (LDL-C):** Known as "Bad Cholesterol," it is the main source of buildup in the arteries (plaque).

**4- Triglycerides (TG):** The most common form of fat stored in the body, used for energy.

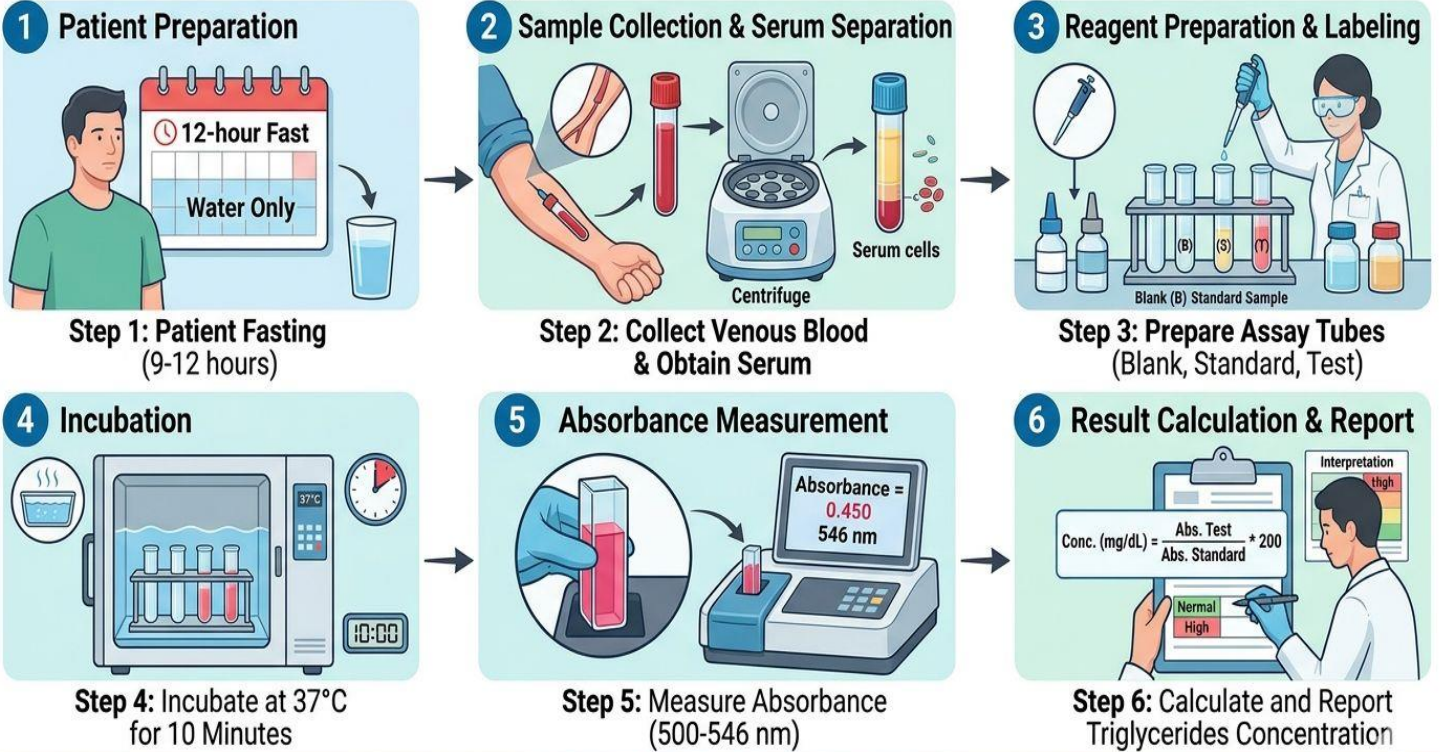
Understanding the balance between these components is more important than any single value alone.



# Practical Part: Laboratory Procedure

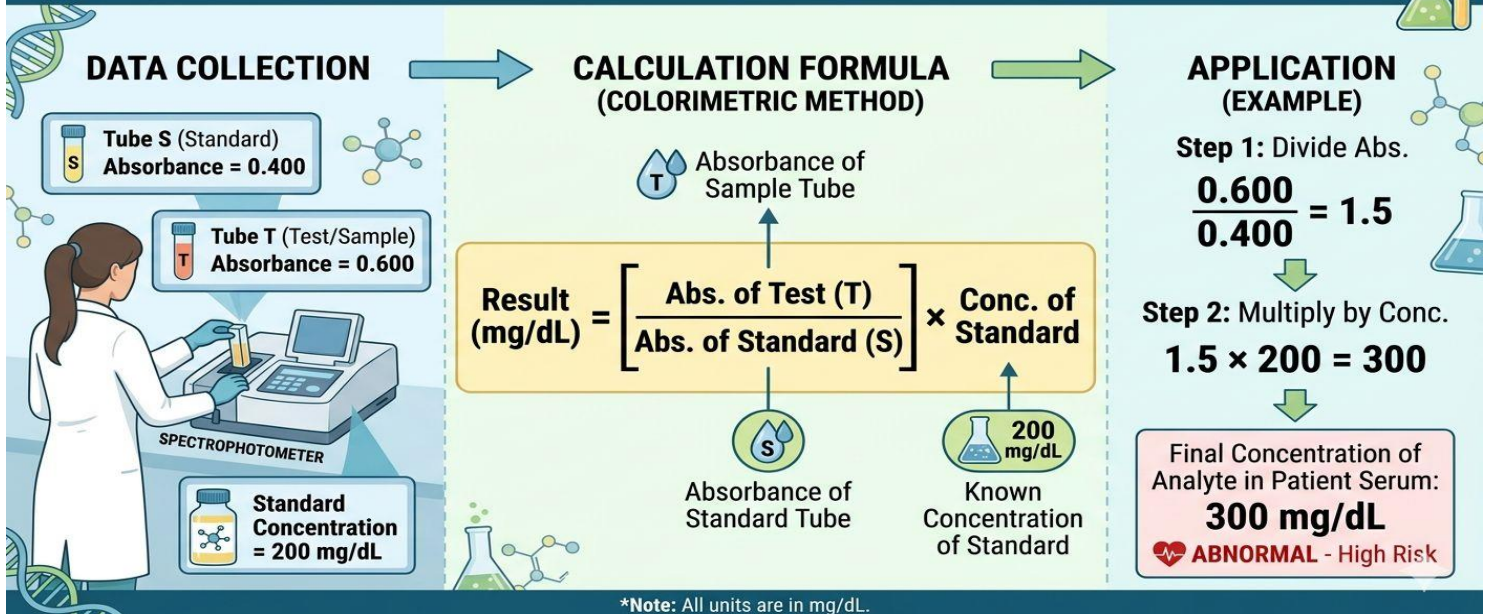
## TRIGLYCERIDES ASSAY PROCEDURE

(Enzymatic colorimetric method)



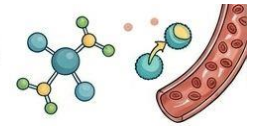
## LABORATORY WORKFLOW

### BIOCHEMISTRY LAB: DETERMINATION OF ANALYTE CONCENTRATION (mg/dL)





# LIPID PROFILE: REFERENCE RANGES & CALCULATION GUIDELINE



| PART 1: NORMAL REFERENCE RANGES<br>(NCEP ATPIII Guidelines) |  |   |                                    |                                       |
|---|--|---|------------------------------------|---------------------------------------|
| <b>Total Cholesterol</b>                                    | <b>&lt; 200 mg/dL</b><br>Desirable         |   |                                    | <b>&gt; 240 mg/dL</b><br>High Risk    |
| <b>LDL Cholesterol (Bad)</b>                                | <b>&lt; 100 mg/dL</b><br>Optimal/Desirable | <b>160-189 mg/dL</b><br>High            |                                    | <b>&gt; 190 mg/dL</b><br>Very High    |
| <b>HDL Cholesterol (Good)</b>                               | <b>&gt; 40 mg/dL</b><br>Desirable Men      | <b>&gt; 50 mg/dL</b><br>Desirable Women | <b>&gt; 60 mg/dL</b><br>Protective | <b>&lt; 40 mg/dL</b><br>Low/High Risk |
| <b>Triglycerides</b>  | <b>&lt; 150 mg/dL</b><br>Desirable         |   |                                    | <b>&gt; 200 mg/dL</b><br>High Risk    |
| <b>VLDL Cholesterol (Derived)</b>                           | <b>2 - 30 mg/dL</b><br>Normal/Desirable    |   |                                    |                                       |

| PART 2: CALCULATION FORMULAS<br>(The Equations)  |  |
|--|--|
| <b>Main Formula 1 (FRIEDEWALD EQUATION)</b>  |  |
| $\text{LDL-C} = \text{Total Cholesterol} - \text{HDL-C} - \left( \frac{\text{Triglycerides}}{5} \right)$ |  |
| Constraint: Valid only if Triglycerides < 400 mg/dL.   |  |
| <b>Secondary Formula 2</b>   |  |
| $\text{VLDL-C} = \frac{\text{Triglycerides}}{5}$   |  |

\*Note: All units are in mg/dL. Consult a healthcare professional for interpretation.

## Importance of Lipid Profile in Rehabilitation & Prosthetics

1. Peripheral Circulation Assessment
2. Stump Wound Healing & Tissue Integrity
3. Prevention of Pressure Ulcers
4. Cardiovascular Endurance for Gait Training
5. Risk Management of Peripheral Vascular Disease (PVD)
6. Weight Management & Socket Fit Optimization
7. Prevention of Secondary Amputations

## Scientific References

1. Tietz Fundamentals of Clinical Chemistry and Molecular Diagnostics (8th Edition). Nader Rifai, Carl Burtis, David Bruns.
2. Clinical Diagnosis and Management by Laboratory Methods. Henry's 23rd Edition.
3. National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III/ATPIII).
4. Friedewald WT, et al. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clinical Chemistry Journal.

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**2025/2026**

**Lecture 6**

**HbA1c**

## Introduction

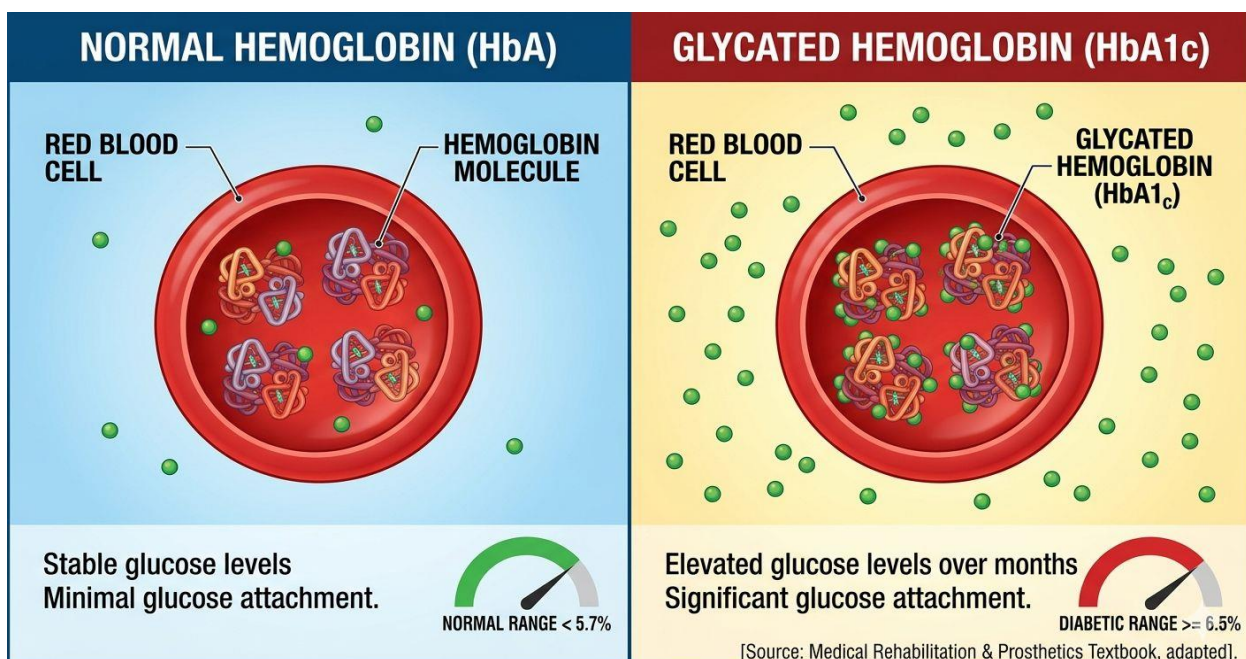
**Glycated Hemoglobin (HbA1c)** is a form of hemoglobin that is chemically linked to a sugar. The HbA1c test measures the average amount of glucose (blood sugar) that has been attached to hemoglobin in the red blood cells over the past 2 to 3 months.

**\*Why 2–3 months?** This corresponds to the average lifespan of a Red Blood Cell (RBC), which is approximately 120 days.

**\*Significance in Rehabilitation:** Monitoring HbA1c is crucial for patients with prosthetic limbs or those undergoing physical therapy, as poorly managed diabetes can lead to poor wound healing, skin breakdown (stump ulcers), and peripheral neuropathy.

### The Glycation Process

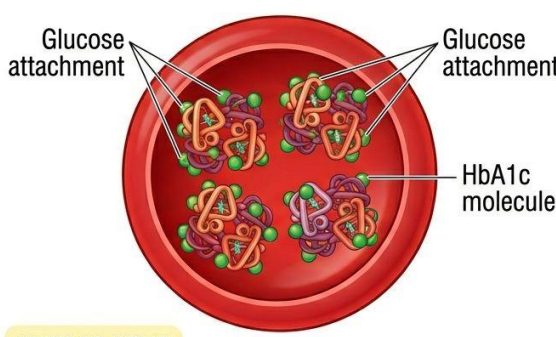
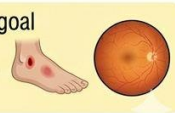
The process is non-enzymatic. When blood glucose levels are high, glucose molecules spontaneously attach to the hemoglobin molecules inside the RBCs. The higher the glucose concentration in the blood, the higher the percentage of hemoglobin that becomes "glycated."



# Clinical Significance & Reference Ranges

According to the American Diabetes Association (ADA) standards, the results are interpreted as follows:

### HBA1C CLINICAL SIGNIFICANCE & REFERENCE RANGES (ADA STANDARDS)

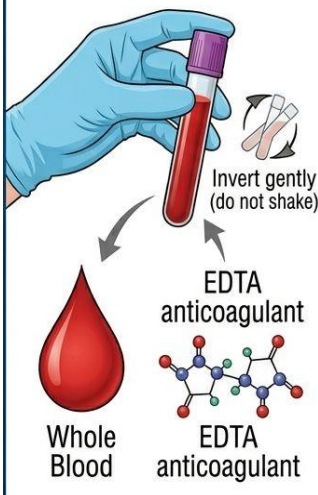
| CATEGORY & REFERENCE RANGES |                 | GLYCATED HEMOGLOBIN (HbA1c) |  |
|-----------------------------|-----------------|-----------------------------|--|
| Category                    | HbA1c Level (%) |                             |  |
| 1 Normal                    | Below 5.7%      | ✓                           |  <p><b>KEY LAB NOTE</b><br/>           Note: For diabetic patients, the general goal is to keep HbA1c below 7% to reduce the risk of microvascular complications (like diabetic foot or retinopathy).</p>  |
| 2 Prediabetes               | 5.7% to 6.4%    | ⚠                           |  |
| 3 Diabetes                  | 6.5% or higher  | ✗                           |  |

[Source: Adapted from American Diabetes Association (ADA) and Medical Rehabilitation & Prosthetics guidelines, <IMAGE 0> reference].

## Practical Part: Laboratory Procedure

### LABORATORY PRACTICE: HbA1c ANALYSIS STEPS & HPLC METHOD

#### 1 SPECIMEN COLLECTION & HANDLING



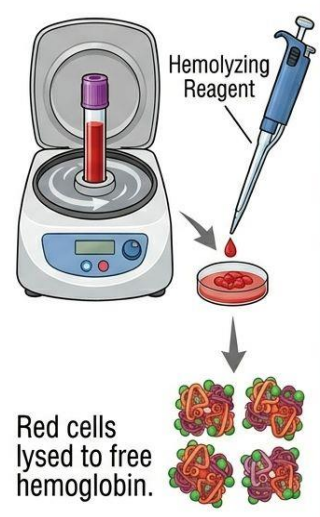
Invert gently (do not shake)

EDTA anticoagulant

Whole Blood

EDTA anticoagulant

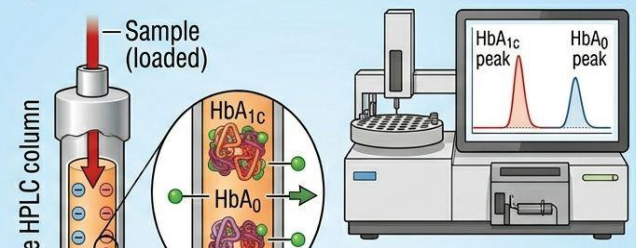
#### 2 HEMOLYSIS (RELEASE OF HEMOGLOBIN)



Hemolyzing Reagent

Red cells lysed to free hemoglobin.

#### 3 HPLC SEPARATION (GOLD STANDARD)



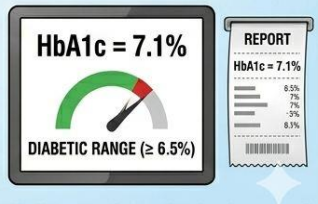
Sample (loaded)

Ion-Exchange HPLC column

Cation-Exchange Resin Column

Hemoglobins separate based on surface charge charge differences.

#### 4 QUANTIFICATION & REPORTING



HbA1c = 7.1%

DIABETIC RANGE (≥ 6.5%)

REPORT

HbA1c = 7.1%

6.5%  
7%  
7.5%  
8.0%

[Source: Adapted from College of Medical Rehabilitation & Prosthetics guidelines and standard references].

## Factors Affecting Results (Interferences)

As students of Rehabilitation and Prosthetics, you must be aware that certain conditions can yield misleading HbA1c results:

- \***Anemia:** Rapid RBC turnover (hemolytic anemia) can falsely lower HbA1c levels.
- \***Recent Blood Loss/Transfusion:** Can alter the "age" of the RBC population.
- \***Hemoglobinopathies:** Variants like HbS (Sickle Cell) or HbC can interfere with some lab methods.

## Scientific Sources

- \*Tietz Textbook of Clinical Chemistry and Molecular Diagnostics.
- \*American Diabetes Association (ADA) - Standards of Medical Care in Diabetes.
- \* IFCC (International Federation of Clinical Chemistry) standardized reference methods.

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**2025/2026**

**Lecture 7**

**Serum Total Protein**

## Introduction

**Serum Total Protein** represents the sum of all proteins present in the liquid portion of the blood (serum). It is a vital indicator of a patient's nutritional status, as well as the functional capacity of the liver and kidneys.

### Types of Serum Proteins

The total protein is primarily composed of two major fractions:

\***Albumin (approx. 60%)**: The most abundant protein, synthesized in the liver. It maintains osmotic pressure and transports hormones and drugs.

\***Globulins (approx. 40%)**: A diverse group of proteins (Alpha, Beta, and Gamma) involved in immunity (antibodies) and blood clotting.

### Biological Functions

Proteins are the "building blocks" of the body. Their main roles include:

\***Osmotic Pressure**: Maintaining the fluid balance between blood vessels and tissues (mainly by Albumin).

\***Transport**: Carrying lipids, vitamins, hormones, and minerals through the blood.

\***Immunity**: Acting as antibodies (Immunoglobulins) to fight infections.

\***Buffering**: Helping maintain the blood's acid-base balance (pH).

### Clinical Significance

\***Hyperproteinemia (High Levels)**: Seen in dehydration, chronic inflammation, or certain cancers like Multiple Myeloma.

\***Hypoproteinemia (Low Levels)**: Seen in malnutrition, liver disease (decreased synthesis), or kidney disease (loss through urine).

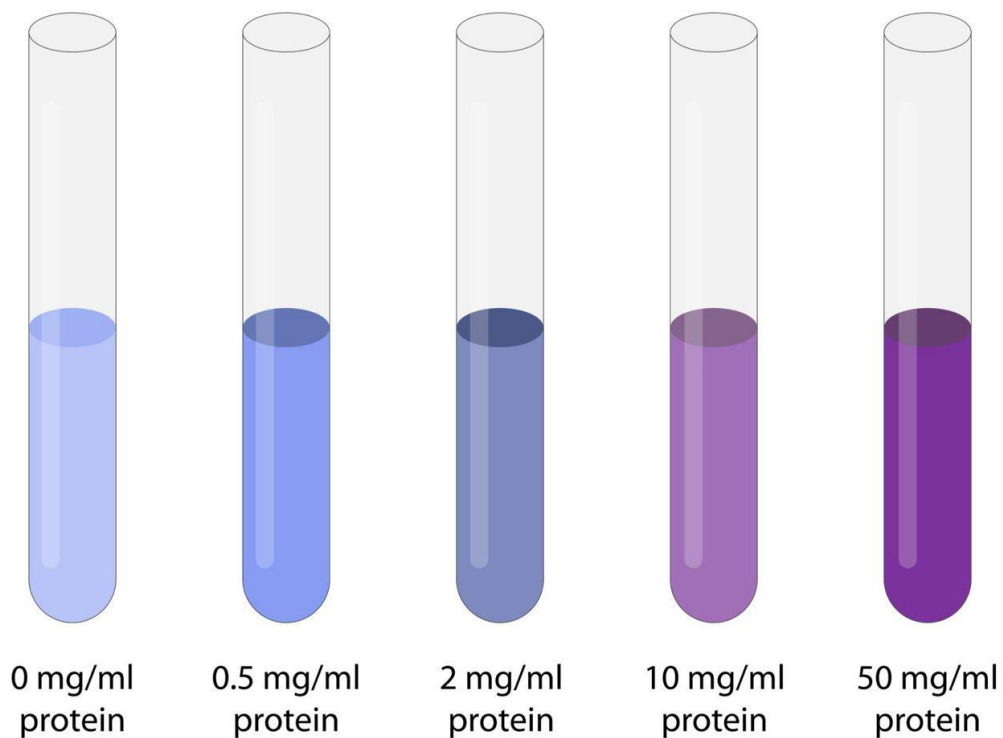
## Practical Part: Biuret Method

The most common manual method for measuring total protein in the lab is the Biuret Method.

### Principle

In an alkaline medium, copper ions ( $\text{Cu}^{2+}$ ) react with the peptide bonds of proteins to form a **violet-colored complex**. The intensity of the color is directly proportional to the concentration of protein in the sample.

### Biuret Test for Proteins

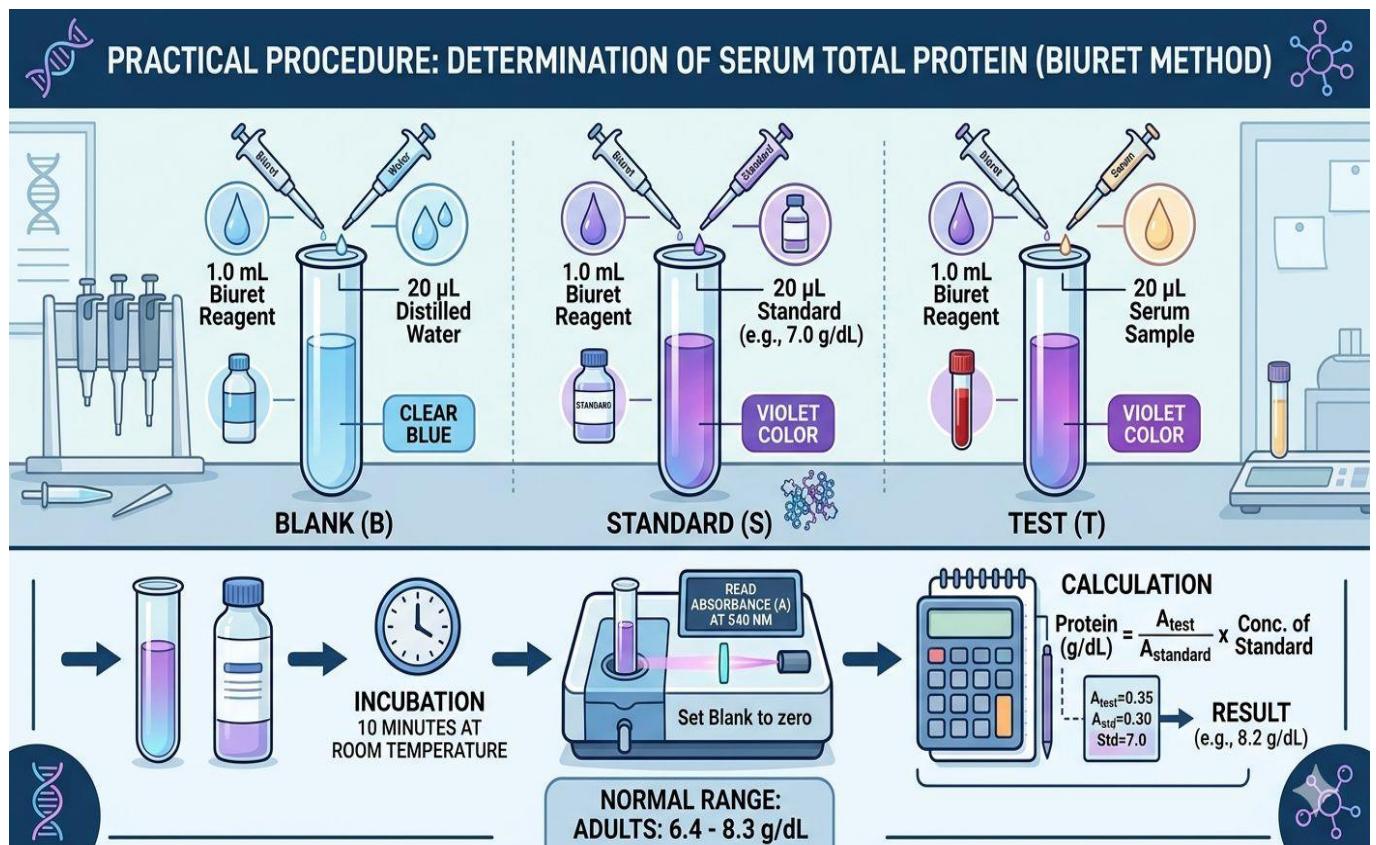


## Reagents & Tools

- 1- **Biuret Reagent:** Contains Copper Sulfate, Sodium Potassium Tartrate, and Sodium Hydroxide.
- 2- **Protein Standard:** A solution with a known concentration (usually 6.0 or 7.0 g/dL.)
- 3- **Spectrophotometer:** Set at a wavelength of 540 nm.
- 4- Test tubes, Micropipettes, and Serum sample.

## Procedure

Label three test tubes: Blank (B), Standard (S), and Test (T)



# SERUM TOTAL PROTEIN CALCULATION GUIDE

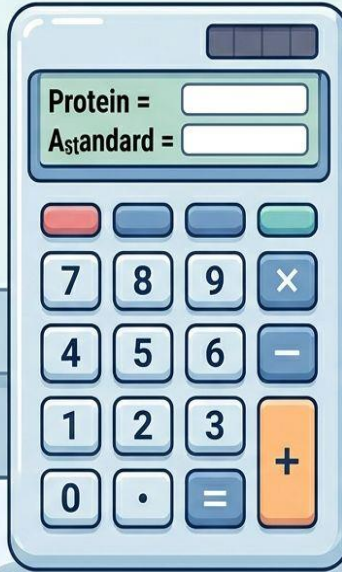
## FORMULA

$A_{\text{test}}$ : Absorbance of Patient Sample

$A_{\text{standard}}$ : Absorbance of Standard Sample

Conc. of Standard: Concentration of Standard (g/dL)

$$\text{Protein (g/dL)} = \left( \frac{A_{\text{test}}}{A_{\text{standard}}} \right) \times \text{Conc. of Standard}$$



## WORKED EXAMPLE

ASSUMED DATA:  
 $A_{\text{test}} = 0.35$   
 $A_{\text{standard}} = 0.30$   
Conc. of Standard = 7.0 g/dL

Substitution:  
 $\left( \frac{0.35}{0.30} \right) \times 7.0$

Intermediate Calculation:  
 $\approx 1.167 \times 7.0$

Final Result:  
 $\approx 8.167$  g/dL

Rounded Final Result  
(e.g., for reporting):  
8.2 g/dL

NORMAL RANGE:  
ADULTS: 6.4 - 8.3 g/dL

## NORMAL RANGE: SERUM TOTAL PROTEIN (ADULTS)

6.4 - 8.3 g/dL

(Reference Interval for Healthy Adults)

Target Demographic:  
Adults

CLINICAL REFERENCE

**Al-Furat Al-Awsat Technical University**

**College of Medical Rehabilitation and Prosthetics**

**Medical Rehabilitation Department and Prosthetics**



Al-Furat Al-Awsat Technical University

**\*\*College of Medical Rehabilitation and Prosthetics\*\***

Assistant Lecturer Shaimaa Al-Hammami

**2025/2026**

**Lecture 8**

**Serum bilirubin**

**(Direct & Indirect)**

## Introduction

**Bilirubin** is a yellow-orange pigment produced from the degradation of hemoglobin after destruction of aged red blood cells. The liver plays an essential role in bilirubin metabolism by conjugating and excreting it into bile.

Serum bilirubin measurement is an important laboratory investigation used in the diagnosis of jaundice, liver diseases, hemolytic disorders, and biliary obstruction.

## Types of Bilirubin

### 1- Indirect Bilirubin (Unconjugated)

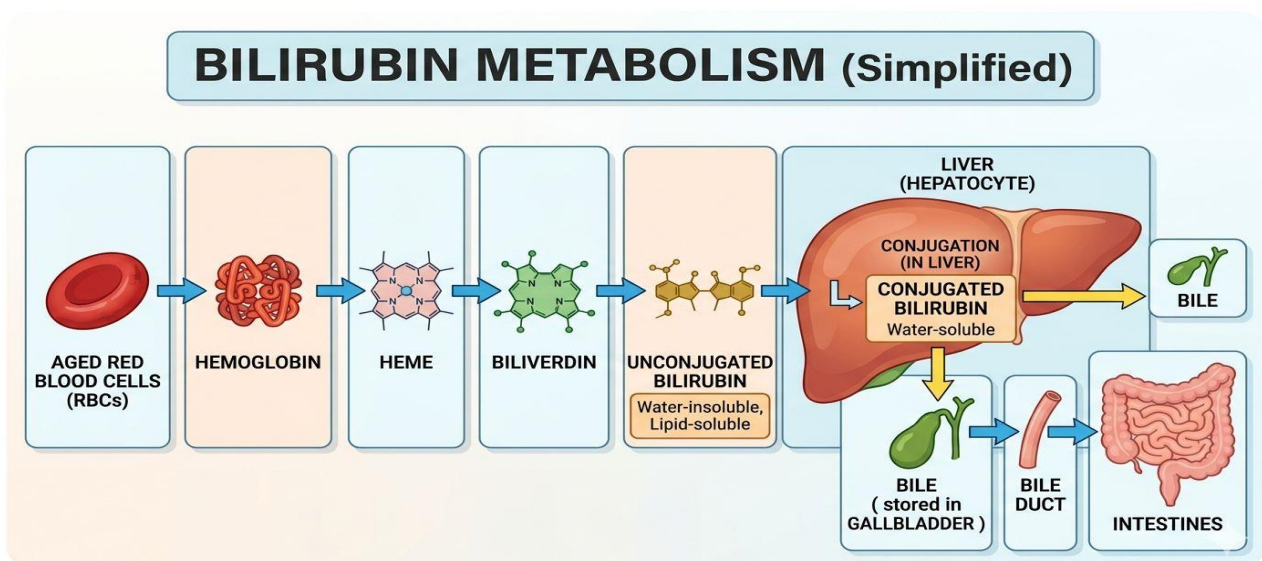
- \*Produced from hemoglobin breakdown.
- \*Lipid soluble.
- \*Bound to albumin in blood.
- \*Cannot pass into urine.

### 2- Direct Bilirubin (Conjugated)

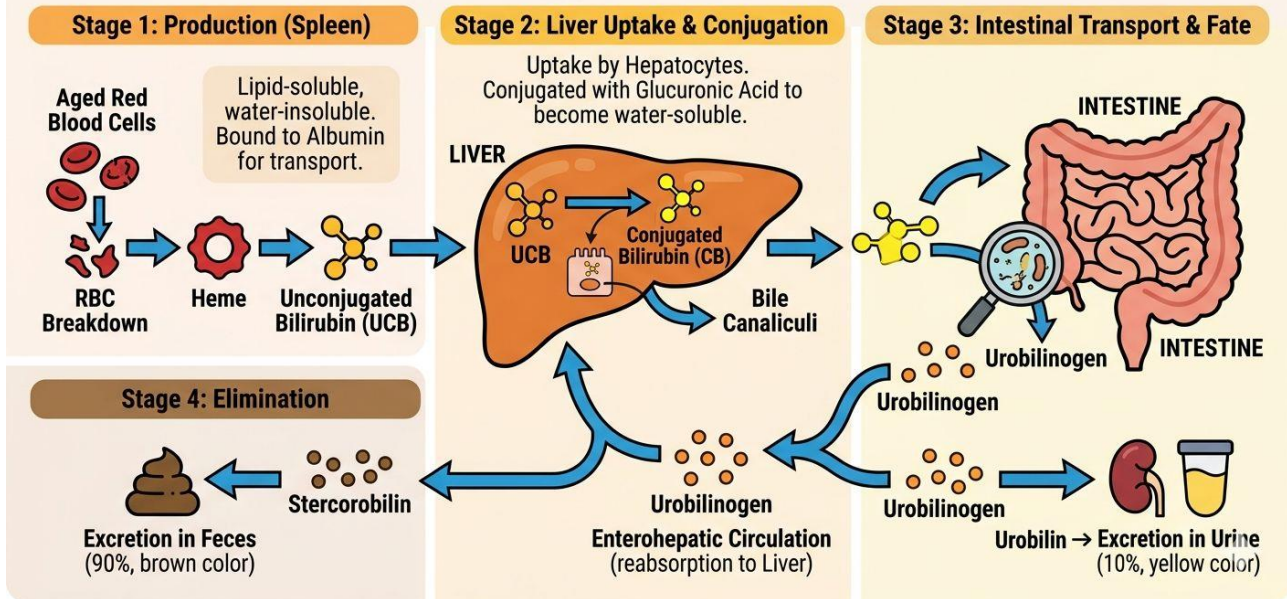
- \*Formed in the liver by conjugation with glucuronic acid.
- \*Water soluble.
- \* Excreted through bile and urine.

## Bilirubin Metabolism

Senescent RBCs → Hemoglobin → Heme → Biliverdin → Unconjugated bilirubin → Liver conjugation → Conjugated bilirubin → Bile → Intestine



# SIMPLIFIED BILIRUBIN METABOLISM PATHWAY



## Laboratory Procedure for Serum Bilirubin

### 1- Sample Preparation

\*Use serum or heparinized plasma.

\*Caution: Avoid hemolysis and protect the sample from light exposure.

### 2- Measure Total Bilirubin (TBIL)

\*Mix the sample with Diazo reagent and an Accelerator (like Caffeine or Methanol).

\*Incubate to allow the reaction to complete.

\*Measure the absorbance at the specified wavelength ( $A_1$ ).

### 3- Measure Direct Bilirubin (DBIL).

\*Mix the sample with Diazo reagent only (without the accelerator).

\*Incubate for a short, precise time (usually 5 minutes).

\*Measure the absorbance ( $A_2$ ).

### 4. Calculation of Indirect Bilirubin (IBIL).

The concentration of Indirect Bilirubin is determined by subtraction:

IBIL = Total Bilirubin – Direct Bilirubin

Alternatively, using absorbance

$$\Delta A(\text{Indirect}) = A_1(\text{Total}) - A_2(\text{Direct})$$

### 5- Requirements & Materials

\*Specimen: Serum or Heparinized Plasma.

\*Critical Note: The sample must be strictly protected from light, as light degrades bilirubin, leading to falsely low results.

\*Reagents:

\*Reagent 1: Sulfanilic Acid.

\*Reagent 2: Sodium Nitrite.

\*Accelerator: Caffeine or Methanol.

\* Equipment: Spectrophotometer (usually set at a wavelength of 546 nm).

### 6- Clinical Interpretation

Normal Reference Ranges:

\*Total Bilirubin: 0.3 - 1.2 mg/dL

\* Direct Bilirubin: 0.0 - 0.3 mg/dL

\* Indirect Bilirubin 0.2-0.9 mg/dL

### 7-Clinical Significance

Increased Indirect Bilirubin Observed in:

\*Hemolytic anemia

\* Neonatal jaundice

\* Gilbert syndrome

Increased Direct Bilirubin Observed in:

\*Hepatitis

\* Obstructive jaundice

\* Liver cirrhosis

## Relation Between Bilirubin and Jaundice

Jaundice is the yellow discoloration of the skin, sclera, and mucous membranes caused by elevated serum bilirubin levels.

Therefore, measurement of direct and indirect bilirubin helps in:

- \*Diagnosis of jaundice
- \*Determination of the type of jaundice
- \*Evaluation of liver function

## Classification of Jaundice According to Bilirubin Changes

### 1- Hemolytic (Pre-hepatic) Jaundice

- \*Caused by excessive destruction of red blood cells.
- \*Leads to increased production of unconjugated (indirect) bilirubin.
- \*The liver cannot conjugate all produced bilirubin.

#### Laboratory Finding:

↑Indirect bilirubin

### 2- Hepatocellular (Hepatic) Jaundice

- \*Caused by liver cell damage such as hepatitis or cirrhosis.
- \*Liver loses ability to conjugate and excrete bilirubin normally.

#### Laboratory Finding:

↑Direct bilirubin

↑Indirect bilirubin

### 3- Obstructive (Post-hepatic) Jaundice

- \*Caused by obstruction of bile ducts.
- \*Conjugated bilirubin cannot reach intestine and leaks into blood.

#### Laboratory Finding:

↑ Direct bilirubin

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**\*\*College of Medical Rehabilitation and Prosthetics\*\***

Assistant Lecturer Shaimaa Al-Hammami

**2025/2026**

**Lecture 9&10**

**Urin glucose and Ketone**

## Introduction

Urinalysis is an important laboratory investigation used to evaluate metabolic and renal disorders.

Among the substances commonly tested in urine are glucose and ketone bodies, which are valuable indicators in diabetes mellitus and other metabolic abnormalities .

### Objectives

By the end of this lecture, students should be able to:

1. Define urine glucose and ketone bodies.
2. Understand the clinical importance of urine glucose and ketone testing.
3. Perform urine glucose testing using Benedict's method or dipstick method.
4. Perform ketone body testing using urine strips.
5. Interpret laboratory results correctly.

### Part One: Urine Glucose Test

#### Definition

Glucose is normally absent or present in very small amounts in urine because the kidneys reabsorb filtered glucose back into the bloodstream. The presence of glucose in urine is called glycosuria or glucosuria .

#### Clinical Causes of Glucosuria

- \*Diabetes mellitus
- \*Pregnancy
- \*Renal glycosuria
- \*Fanconi syndrome
- \*Stress and excessive carbohydrate intake

#### Principle of the Test



Most urine dipsticks use the glucose oxidase enzymatic reaction, which specifically detects glucose in urine .

## Materials Required

- \*Fresh urine sample
- \*Urine dipstick strips
- \*Gloves
- \*Test tube (if Benedict's test is used)
- \*Benedict's reagent
- \*Pipette


## Procedure (Dipstick Method)

1. Wear gloves.
2. Mix the urine sample gently.
3. Dip the reagent strip into urine.
4. Remove excess urine.
5. Wait for the specified time according to manufacturer instructions.
6. Compare the strip color with the color chart.

## Benedict's Test Procedure

1. Place 5 mL of Benedict's reagent in a test tube.
2. Add 8–10 drops of urine.
3. Heat gently for 2 minutes.
4. Observe the color change.

| INTERPRETATION OF BENEDICT'S TEST |           |
|-----------------------------------|-----------|
| Result                            | Color     |
| Negative                          | Blue      |
| Trace                             | Green     |
| +                                 | Yellow    |
| ++                                | Orange    |
| +++                               | Brick red |



## **Clinical Significance**

**Glucosuria may indicate:**

- \*Diabetes mellitus**
- \*Hyperglycemia**
- \*Renal tubular disorders**

However, urine glucose testing alone is not sufficient for diagnosing diabetes and should be supported by blood glucose testing .

## **Part Two: Urine Ketone Test**

### **Definition**

Ketone bodies are produced when the body breaks down fats for energy instead of glucose. The major ketone bodies are:

- \*Acetoacetic acid**
- \*Acetone**
- \*Beta-hydroxybutyric acid**

### **Causes of Ketonuria**

- \*Uncontrolled diabetes mellitus**
- \*Starvation or fasting**
- \*Severe vomiting**
- \*Low-carbohydrate diets**
- \*Excessive exercise**

### **Principle of Ketone Test**

**{Acetoacetic Acid} + {Nitroprusside} = {Purple Complex}**

The urine ketone strip contains sodium nitroprusside, which reacts mainly with acetoacetic acid to produce a purple color .

## Materials Required

- \*Fresh urine sample
- \*Ketone reagent strips
- \*Gloves

## Procedure

- 1- Dip the ketone strip into fresh urine.
- 2- Remove excess urine.
- 3- Wait for 30–60 seconds.
4. Compare the color with the standard chart.



## Clinical Importance

**Ketonuria is commonly associated with:**

- \*Diabetic ketoacidosis (DKA)
- \*Starvation
- \*Prolonged vomiting
- \*Severe dehydration

## Precautions

- \*Use fresh urine samples.
- \*Read results within the recommended time.
- \*Avoid contamination of strips.
- \*Store strips away from moisture and heat.
- \*Wear personal protective equipment during testing.

## Conclusion

Urine glucose and ketone tests are simple, rapid, and valuable laboratory procedures used in detecting diabetes mellitus and metabolic disturbances. Accurate performance and interpretation of these tests are essential in clinical laboratory practice .

## References

- 1- MedlinePlus – Glucose in Urine Test<sup>[OBJ]</sup>
- 2- MedlinePlus – Ketones in Urine Test<sup>[OBJ]</sup>
- 3- Merck Manual – Urinalysis and Urine Culture<sup>[OBJ]</sup>
- 4- Mayo Clinic – Urinalysis<sup>[OBJ]</sup>
5. NCBI Bookshelf – Urinalysis<sup>[OBJ]</sup>