Glucose measurement by:
enzymatic method in biochemical lab

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Introduction

- Glucose measurement is one of the most frequently performed tests in clinical biochemistry laboratories. It is essential for diagnosing, monitoring, and managing conditions such as diabetes mellitus, hypoglycemia, metabolic disorders, and endocrine diseases.
- **Sources of blood glucose:**
- - Dietary carbohydrates
- - Glycogen breakdown in the liver
- - Gluconeogenesis

- The body tightly regulates glucose to maintain a normal range and ensure adequate cellular energy supply.

Hormonal regulation of blood glucose

- Blood glucose levels are mainly controlled by two main hormones:

- **Insulin (from β-cells):**
- - Decreases blood glucose
- - Promotes glucose uptake by muscles and fat
- - Converts excess glucose to glycogen (liver, muscles)
- - Stimulates fat storage

- **Glucagon (from α-cells):** *with counter-regulatory hormones as epinephrine, cortisol and growth hormone*
- - Increases blood glucose
- - Stimulates glycogenolysis and gluconeogenesis in the liver

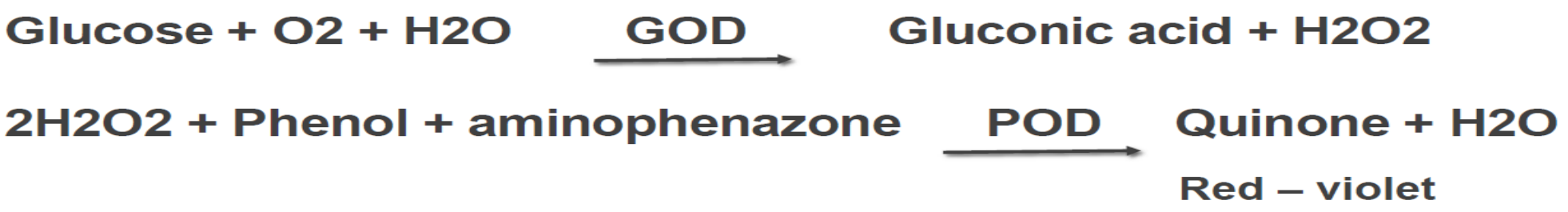
Specimen Types and Handling

- - **Serum:** if sample collected in gel separator tube it should be processed within 30 minutes to reduce risk of glycolysis
- - **Plasma:** preferred sodium fluoride tube to inhibit RBC enzyme activity and prevent glycolysis
- Whole blood is less accurate and not preferred
- - careful handling to avoid hemolysis

Principle of Glucose Measurement

- Enzymatic Colorimetric (**Glucose Oxidase–Peroxidase Method**):
- - Glucose oxidase (**GOD**) converts glucose → gluconic acid + H_2O_2
- - Peroxidase (**POD**) reacts with H_2O_2 and chromogens (phenol + 4-aminophenazone) to form a red-violet quinone dye
- - Read color intensity at 500–505 nm using a spectrophotometer

Reaction:



Reagents used in glucose measurement

- - Working reagent (buffer + enzymes + chromogens)

Tris Buffer 92mmol/L at PH = 7.4, Phenol 0.3mmol/L, Glucose oxidase (GOD) and 4-Aminophenazone.

- - Glucose standard (100 mg/dL)
- - Distilled water (for blank)

Procedure (GOD-POD Method)

- Prepare three tubes:

Tubes / Solutions	Blank	Standard	Serum
Working Reagent	1 mL	1 mL	1 mL
Standard	-	10 µL	-
Sample (Serum)	-	-	10 µL

- Steps:
- - Mix and incubate for 10 minutes at 37°C (or 20 minutes at room temp)
- - Measure absorbance at ~500-505 nm against the blank

CALCULATIONS

$$\frac{\text{(A)Sample}}{\text{(A)standard}} \times 100(\text{std conc}) = \text{mg/dL glucose}$$

Conversion factor: mg/dL x 0.0555=mmol/L

A= absorbance

Std conc =standard concentration

Example:

- (A) sample = 0.42
- (A) standard = 0.35

Glucose = 0.42 / 0.35 × 100 = **120 mg/dL**

- In mmol/L = 120 × 0.0555 = 6.66 mmol/L

Clinical Interpretation

- **1- Fasting State:** No food or drink (only water) for 8-12 hours =70–10 **mg/dL**
- ≥126 mg/dL → Diabetes

- **2- Postprandial State:** 2 hours after the meal or after oral glucose (**OGTT**)= <140 mg/dL
- ≥200 mg/dL → Diabetes

- **3- Random glucose** ≥200 mg/dL with symptoms → Diabetes

- <70 mg/dL → **Hypoglycemia** (requires evaluation)

- **Unit conversion:**
- - mmol/L → mg/dL: × 18
- - mg/dL → mmol/L: × 0.0555 (**or** /18)

Glucometer vs Laboratory Methods

- **Glucometer:** Low cost, portable, home or clinic use
- - Capillary blood
- - Results in seconds
- - Moderate accuracy ±15% (which is accepted)
- - Not used for diagnosis

- **Laboratory analyzer:** Higher cost, requires lab facilities and trained personnel
- - Venous plasma/serum
- - results need more time (minutes)
- - High accuracy
- - Used for diagnosis

Scenario Question:

- A 45-year-old patient comes to the lab **fasting** at the morning for a routine checkup. You are performing the glucose assay using the GOD-POD method.
- The **glucose standard** (100 mg/dL) gives an **absorbance of 0.50**.
- The **patient's serum sample** gives an **absorbance of 0.75**.
- **Tasks:**
- 1-Calculate the patient's **blood glucose concentration** in **mg/dL**.
- 2-Convert the result to **mmol/L**.
- 3-Based on the calculated value, interpret the patient's glucose result

Good luck