Glucose measurement by
Glucose oxidase in biochemical lab

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Introduction

- **Blood glucose measurement is one of the most important biochemical tests used in the diagnosis and monitoring of diabetes mellitus.The glucose oxidase (GOx) method is one of the most widely used enzymatic colorimetric methods because it is sensitive, accurate, inexpensive, and specific for glucose, making it the standard method in many laboratories.**

- Sources of blood glucose:
- – Dietary carbohydrates
- – Glycogen breakdown in the liver
- – Gluconeogenesis

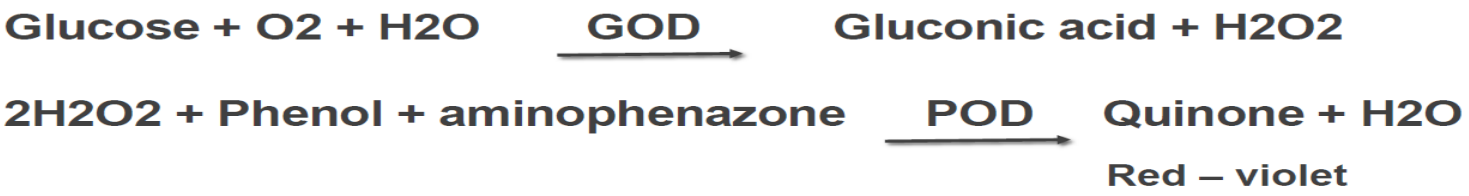
Specimen Types and Handling

- - **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(Glucose oxidase (GOx) catalyzes oxidation of β-D-glucose)
- - 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

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)**

Clinical Significance

Hyperglycemia (High glucose):

Seen in: Diabetes mellitus, Cushing's syndrome, stress, infections

Causes: Polyuria, polydipsia, dehydration, blurred vision

Chronic elevation → nephropathy, retinopathy, neuropathy

Hypoglycemia (Low glucose):

Causes: Excess insulin, skipping meals in diabetics, liver disease

Symptoms: Sweating, tremor, confusion, weakness

Severe hypoglycemia can be dangerous and needs urgent correction

Good luck