

# Quantitative estimation of glucose by enzymatic method

BCH302 [Practical]

# Methods of estimation the reducing sugar content in solution :

- **There are three main methods of estimation the reducing sugar content in solution :**

1. Reduction of cupric to cuprous salts.
2. Reduction of ferricyanide to ferrocyanide.
3. Enzymatic method.

- **Note:**

Enzymatic method is the most commonly used in clinical laboratories for glucose estimation .

# Methods of estimation the reducing sugar:

## 1- Reduction of cupric to cuprous salts:

- Reducing sugars contains an aldehyde or keto groups reduced alkaline copper to Cuprous oxide.
- Cuprous oxide is allowed to react with phosphomolybdate solution which is reduced and forms **blue color.**
- The **intensity** of color is measured on colorimeter against standard.

## 2- Reduction of ferricyanide to ferrocyanide:

- Reduction of ferricyanide to ferrocyanide by reducing sugars in **alkaline solution** .
- In presence of zinc ions , the ferrocyanide formed is precipitated as a zinc complex.

## 3- Enzymatic method:

- Glucose is commonly measured using an **enzyme** to convert the glucose to a product that can be easily detected.
- Common enzymes used are **glucose oxidase, glucose dehydrogenase and hexokinase.**

# Determination of blood glucose:

- Glucose is a major carbohydrates present in the peripheral blood.
- The oxidation of glucose is the major source of cellular energy in the body.
- Glucose determination are run primarily to aid in the diagnosis and treatment of diabetes-mellitus.

- Range of expected values in serum (Normal range):

**70-105 mg/dl (3.9-5.8 mmol/L) - Fasting**

# Practical part

# Experiment 1 : Estimation of blood glucose level by Glucose oxidase

## Objective:

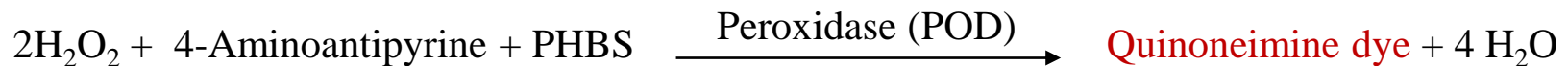
- Quantitative determination of glucose in serum using a modified glucose oxidase (GOD) / Trinder method.

## Principle:

- The enzymatic reaction sequence employed in the assay of glucose is as follows:**
  - Glucose oxidase converts **glucose**, in the presence of oxygen, to **gluconic acid** and hydrogen peroxide:



- The hydrogen peroxide is oxidatively coupled with 4-aminoantipyrine and p-hydroxybenzene sulfate (PHBS) in the presence of peroxidase to form a stable red **quinoneimine dye**:



- The quinoneimine dye has an absorption maximum at **510nm**. The amount of colour produced is **directly proportional** to the **glucose content** of the sample.

# Experiment 1 : Estimation of blood glucose level by Glucose oxidase

## Method:

1. Pipette to clean 3 cuvettes:

	<b>Blank</b>	<b>Standard</b>	<b>Test</b>
Glucose oxidase liquid reagent	1 ml (1000 $\mu$ l)	1 ml (1000 $\mu$ l)	1 ml (1000 $\mu$ l)
<b>Pre-warm at 37 °C and add:</b>			
Glucose standard	-	0.01 ml (10 $\mu$ l)	-
Sample	-	-	0.01 ml (10 $\mu$ l)

2. Mix and incubate at 37 °C for 15 min.

3. Read the absorbance of sample and standard at **510nm** against blank.

# Experiment 1 : Estimation of blood glucose level by Glucose oxidase

## Results:

- Use the absorbance measurement of the STANDARD and TEST to calculate glucose values as follows:

$$\frac{A(\text{test})}{A(\text{standard})} \times \text{concentration of standard (mg/dl)} = \text{glucose concentration in test (mg/dl)}$$

- **Example:**

$$A(\text{standard}) = 0.325$$

$$A(\text{test}) = 0.300$$

So:

$$\frac{0.300}{0.325} \times 100 \text{ mg/dl} = 92 \text{ mg/dl} \quad \rightarrow \text{Normal}$$