# 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:**

1- Glucose oxidase converts glucose, in the presence of oxygen, to gluconic acid and hydrogen peroxide:

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{GOD}} \text{Gluconic acid} + H_2O_2$$

2- 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:

$$2H_2O_2 + \text{4-Aminoantipyrine} + \text{PHBS} \xrightarrow{\text{Peroxidase (POD)}} \text{Quinoneimine dye} + 4\ H_2O$$

- 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:

| | Blank | Standard | Test |
|---|---|---|---|
| Glucose oxidase liquid reagent | 1 ml (1000µl) | 1 ml (1000µl) | 1 ml (1000µl) |
| **Pre-warm at 37 °C and add:** | | | |
| Glucose standard | - | 0.01 ml (10µl) | - |
| Sample | - | - | 0.01 ml (10µl) |

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:

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

- **Example**:

A (standard) = 0.325
A(test) = 0.300
So:

$\frac{0.300}{0.325}$ X 100 mg/dl = 92 mg/dl ➔ Normal