ESTIMATION OF PLASMA GLUCOSE BY GOD -POD METHOD

Learning Objectives

1. **Principle**
2. **Reaction**
3. **Reagents**
4. **Procedure**
5. **Data**
6. **Calculation**
7. **Result**
8. **Reference Range**
9. **Interpretation**
10. **Other Methods**

1. Principal

- Glucose oxidase (GOD) oxidizes the specific substrate β-D- glucose to gluconic acid and hydrogen peroxide (H_2O_2) is liberated. Peroxidase (POD) enzyme acts on hydrogen peroxide to liberate nascent oxygen (O_2), then nascent oxygen couples with 4- amino antipyrine and phenol to form red quinoneimine dye.

- The intensity of the colour is directly proportional to the concentration of glucose present in plasma. The intensity of colour is measured by colorimeter at 530 nm or green filter and compared with that of a standard treated similarly. Final colour is stable for at least 2 hours if not exposed to direct sunlight.

2. Reaction

Reaction: -

1. Glucose + O_2 + H_2O —(*Glucose Oxidase*)→ Gluconic acid + H_2O_2.

2. H_2O_2 + Phenolic Compounds + 4-aminoantipyrine —(*Peroxidase*)→ Quinoneimine (Red colour complex) + H_2O

3. Reagents

1. Glucose colour reagent; it contains GOD, POD, 4- amino antipyrine, phenol & phosphate buffer (pH 7.5)
2. Glucose standard solution, Concentration = 100 mg/dl. 100 mg of anhydrous glucose is dissolved in 100 ml of distilled water

4. Procedure

- Pipette into clean, dry test tube labelled as Blank (B), Standard (S) and Test (T).
- Then add the solution in each of test tubes separately as shown in table below.

	BLANK	STANDARD	TEST
Glucose colour Reagent	**1000 µl**	**1000 µl**	**1000 µl**
Distilled Water	**10 µl**	**-----**	**-----**
Standard	**-----**	**10 µl**	**-----**
Plasma	**-----**		**10 µl**
Mix thoroughly and keep the tubes at 37˚C for 15 minutes.			
OD at 530 nm	0.02	0.45	0.58

5. Data

- Plasma Glucose standard concentration is 100 gm/dl

6. CALCULATION`

Concentration of Glucose $= \frac{O.D.of\ Test - O.D.of\ Blank}{O.D.\ of\ Standard - O.D.of\ Blank} \times Concentration\ of\ Standard$

7. Result

- Plasma glucose concentration in given unknown blood sample =--------mg/dl.

8. Normal Range

	Fasting	After eating	2-3 hrs after eating
Normal	80 - 100	170 - 200	120 - 140
Pre Diabetic	101 - 125	190 - 230	140 - 160
Diabetic	126+	220 - 300	200+

9. Interpretation

- **Hyperglycemia:**
- **It is found in following conditions**
- I. Physiological**:**
- 1. Alimentary : After high carbohydrate diet
- 2. Emotional: Stress, anger, anxiety etc.
- II. **Pathological:**
- 1. Diabetes mellitus
- 2. Hyperadrenalism
- 3. Hyperpituitarism

- **Hypoglycemia:**
- **It is found in following conditions:**
- **I. Physiological:**
  - During starvation
  - After Severe Exercise
- II. **Pathological:**
  - Prolonged fasting
  - Due to excess of insulin e.g.
    - Excessive dose of insulin
    - No food intake after insulin administration
    - Tumours of pancreas (insulinoma)
  - Glycogen storage disease
  - Hypoactivity of adrenal and pituitary gland

Other Methods

- Modified Folin Wu method
- O-Toluidine method
- Hexokinase method

Thank you