# Biochemistry and Immunology Lab (BBT209)

**Experiment 2a**
**Estimation of Glucose in Grapes by enzymatic method using a standard curve**

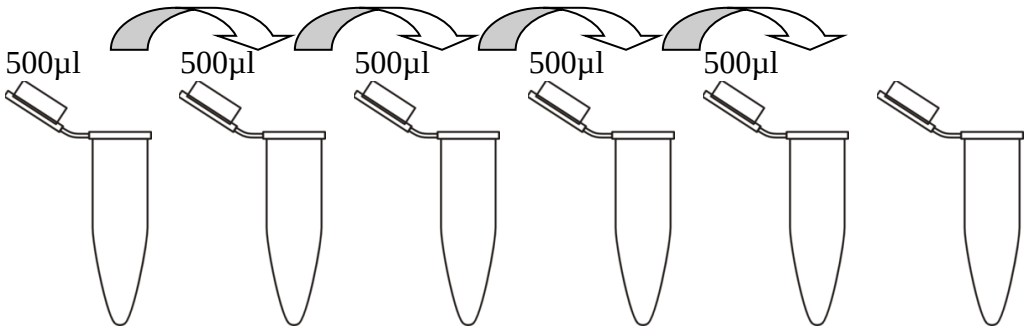
**Background:**
Glucose is the single most important energy source of the brain and the primary energy supplier of the body.
Numerous foods function as sources of glucose. Most dietary carbohydrates contain glucose, either as their only building blocks, as in starch and glycogen or together with other monosaccharide. Since ancient times, fruits have been an important part of human diet, supplying us with sugars, fibres, vitamins, minerals, water and even fat. Despite fructose being the principal sugar in fruits, they also contain significant amount of glucose, which is important for people suffering from fructose malabsorption, diabetes etc. Some fruits containing more fructose than glucose, e.g. apples, pears, watermelon may be more of a problem than ones with less fructose than glucose like berries, bananas and oranges.

**Principle:**
This procedure is based on Trinder method in which aldehyde group of glucose is oxidized by glucose oxidase (GOD) to gluconic acid. In the presence of peroxidise (POD), the formed hydrogen peroxide reacts with chromogenic oxygen acceptor, phenol-aminophenazone (4-AAP) and resultant is the red coloured quinine. The intensity of the colour produced is directly proportional to glucose concentration.
Glucose+$O_2$+ $H_2O$→ Gluconic acid +$H_2O_2$
2$H_2O_2$ +4-AAP+ Phenol→ Quinoneimine dye (red coloured) +4$H_2O$

**Reagents:**

| | | |
|---|---|---|
| Buffer R | TrispH7.4 | 92 mmol/l |
| | Phenol | 0.3 mmol/l |
| | Glucose oxidase (GOD) | 15000 U/l (unit/liter) |
| | Peroxidase (POD) | 1000 U/l |
| | 4-aminophenazone (4-AP) | 2.6 mmol/l |

**Procedure:**
**Specimen:** grape juice
**Standard preparation**: Using the given glucose standard, prepare 5 standards of different concentrations (25, 50, 100, 200 and 400 mg/dl) using serial dilution from a stock solution of 800 mg/dl.




| | **Desired Concentration (mg/dl)** | **Dilution** | **Volume of D-Glucose solution (µl)** | **$dH_2O$ (µl)** | **Total volume (ml)** |
|---|---|---|---|---|---|
| 5. | 400 | 1:2 | 500 | 500 | 1.0 |
| 4. | 200 | 1:2 | 500 | 500 | 1.0 |
| 3. | 100 | 1:2 | 500 | 500 | 1.0 |
| 2. | 50 | 1:2 | 500 | 500 | 1.0 |
| 1. | 25 | 1:2 | 500 | 500 | 1.0 |
| Blank | 0 | 1 | 0 | 1000 | 1.0 |

**Sample preparation**:
For Sample A prepare a .....X dilution and Sample B with a .....X dilution of the fruit juice by diluting the juice in distilled $H_2O$.

**Assay preparation:**
Take 8 test tubes and level them as B (Blank), Std1, Std2, Std3, Std4, Std5, Smpl A and Smpl B. Prepare the test tubes as shown in the table below:

| | **Blank (B)** | **Standard 1** | **Standard 2** | **Standard 3** | **Standard 4** | **Standard 5** | **Sample A** | **Sample B** |
|---|---|---|---|---|---|---|---|---|
| **RT** | 1.0ml | 1.0ml | 1.0ml | 1.0ml | 1.0ml | 1.0ml | 1.0ml | 1.0ml |
| **Specimen** | 10µl of distilled water | 10µl of 25 mg/dl solution | 10µl of 50 mg/dl solution | 10µl of 100 mg/dl solution | 10µl of 200 mg/dl solution | 10µl of 400 mg/dl solution | 10µl of …… X diluted fruit juice | 10µl of ……X diluted fruit juice |
| **Absorbance** | | | | | | | | |

1. Mix the contents well and incubate for 10 minutes at 37ºC or 20 minutes at room temperature.
2. Measure the absorbance of the specimen and standard against the blank at 505nm using Biochemistry analyzer.

**Experiment 2b**

**Estimation of glucose in plasma by enzymatic method using semi-automated Biochemistry Analyzer**

**Background:**

Glucose is the major carbohydrate present in the peripheral blood. Oxidation of glucose is the major source of cellular energy in the body. Glucose derived from dietary sources is converted to glycogen for storage in the liver or to fatty acids for storage in adipose tissue. The concentration of glucose in the blood is controlled within narrow limits by many hormones, the most important of which are produced by the pancreas. The most frequent causes of hyperglycemia are diabetes mellitus resulting from deficiency in insulin secretion or action. A number of secondary factors also contribute to elevated blood glucose levels which lead to diseases. These include pancreatitis, thyroid dysfunction, renal failure and liver diseases. Hypoglycemia is less frequently observed. A variety of factors may cause low glucose level which causes insulinomia, hypopituitarism or insulin induced hypoglycaemia etc.

**Principle:**

This procedure is based on Trinder method in which aldehyde group of glucose is oxidized by glucose oxidase (GOD) to gluconic acid. In the presence of peroxidise (POD), the formed hydrogen peroxide reacts with chromogenic oxygen acceptor, phenol-aminophenazone (4-AAP) and resultant is the red coloured quinine. The intensity of the colour produced is directly proportional to glucose concentration.

Glucose+$O_2$+ $H_2O$→ Gluconic acid +$H_2O_2$

$2H_2O_2$ +4-AAP+ Phenol→ Quinoneimine dye (red coloured) +$4H_2O$

**Reagents:**

| | | |
|---|---|---|
| Buffer R | Tris pH7.4 | 92 mmol/l |
| | Phenol | 0.3 mmol/l |
| | Glucose oxidase (GOD) | 15000 U/l (unit/liter) |
| | Peroxidase (POD) | 1000 U/l |
| | 4-aminophenazone (4-AP) | 2.6 mmol/l |

**Procedure:**

**Specimen:** Plasma. Blood collected individual with good health condition.

**Specimen preparation**: Blood must be collected in an EDTA (Ethylene diamine tetra acetic acid) tube (tube with anticoagulant) and normal saline. Specimen may be stored at 2-8ºC prior to analysis. Cells are removed from plasma by centrifugation.

Prepare the 3 test tubes with blank, standard and sample, as shown below:

| | **Blank** | **Standard** | **Sample** |
|---|---|---|---|
| **R** | 1.0 ml | 1.0 ml | 1.0 ml |
| **Standard** | - | 10 µl | - |
| **Sample** | - | - | 10 µl |
| **$dH_2O$** | 10 µl | - | - |
| **Absorbance** | | | |

1. Mix well and incubate for 10 minutes at 37 ºC or 20 minutes at room temperature.
2. Measure the absorbance of the specimen and standard against reagent blank at 505nm using Biochemistry analyzer

**Calculation:** Calculate the glucose concentration by using following formula:

**Glucose concentration (mg/dl)** = (Absorbance of Sample/Absorbance of standard) x [Concentration of the standard]

**Expected value (plasma):**

- A normal fasting (no food for 8-10 hours) blood sugar level is between 70-100 mg/dl (3.88 mmol-5.55 mmol).
- A normal blood sugar level two hours taking 75 g glucose or meal after eating is less than 140 mg/dl.