Welcome

Biochemistry II

- Serum
- Clotted Blood
- Plasma
- WBCs & Platelets
- RBCs
Blood glucose

Glucose is the main sugar present in blood

O=C\H
H-C-OH
HO-C-H
H-C-OH
H-C-OH
CH_2OH
Glycolysis in RBCs may affect blood glucose determination:

- Glucose disappears rapidly from whole blood on standing.

- Glucose → glycolysis → Lactic acid hence, up to about 10 mg/dl may be lost/hour.

- This can be prevented by adding sodium fluoride (NaF) to the blood sample.
Methods used for determination of blood glucose:

Reducing methods
- Phosphomolybdic Acid method
- O-Toluidine method

Enzymatic methods
- Glucose Oxidase method
- Hexokinase method

Apparent blood glucose
- > 30 mg/dl

More accurate
• Why is apparent glucose value > true glucose value by 30 mg/dl?

• Because when using reducing methods, other reducing compounds in blood (as glutathione, glucouronanic acid, uric acid and ascorbic acid) interfere with glucose.
Enzymatic Methods

Glucose oxidase method:

**Principle:**

1. Glucose + O$_2$ + H$_2$O → Gluconic acid + H$_2$O$_2$
2. H$_2$O$_2$ + O-acceptor + Coloring agent (4-aminophenazone) (Phenol) → Coloured chromogen (Rose colour)

Glucose oxidase

Peroxidase
<table>
<thead>
<tr>
<th>Reagent</th>
<th>Blank</th>
<th>Standard</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sample</strong></td>
<td>......</td>
<td>......</td>
<td>0.1 ml</td>
</tr>
<tr>
<td><strong>Standard (100 mg/dl)</strong></td>
<td>......</td>
<td>0.1 ml</td>
<td>......</td>
</tr>
<tr>
<td><strong>Working reagent</strong></td>
<td>1.0 ml</td>
<td>1.0 ml</td>
<td>1.0 ml</td>
</tr>
</tbody>
</table>

Mix and incubate for 10 min. in W.B. (37°C) and measure absorbance of Test & Std. at 510 nm.
Calculation:

Conc. of glucose (mg/dl) = \( \frac{\text{Abs. of T}}{\text{Abs. of St.}} \times \text{Conc. of st.} \) (100 mg/dl)
## Blood Glucose Level

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Borderline</th>
<th>Diabetic</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fasting</strong> <strong>(8-10 hours)</strong></td>
<td>&lt; 100 mg/dl</td>
<td>100-125 mg/dl</td>
<td>&gt;126 mg/dl</td>
</tr>
<tr>
<td><strong>Postprandial</strong> <strong>(2 hours after balanced meal)</strong></td>
<td>&lt; 140mg/dl</td>
<td>140-199 mg/dl</td>
<td>&gt;200mg/dl</td>
</tr>
</tbody>
</table>
**Interpretation**

**Physiological:**

FBG due to increased circulating epinephrine as in strenuous exercise and strong emotion (fear).

**Pathological**

Diabetes mellitus (DM)