GLUCOSE ESTIMATION METHOD
INTRODUCTION:

• Glucose is a monosaccharide.
• It is central molecule in carbohydrate metabolism.
• Stored as glycogen in liver and skeletal muscle.
Entry of glucose into the cell

Two specific transport system are used:

1. Insulin-independent transport system:
   • Carrier mediated uptake of glucose
   • Not dependent on insulin.
   • Present in hepatocytes, erythrosytes & brain.

2. Insulin dependent transport system:
   • Present in Skeletal muscle.
• For glucose estimation from any material, blood is collect in fluoride containing vial.
• Fluoride inhibit glycolysis by inhibiting enolase enzyme.
• In CSF, bacteria & other cells are also present so analyzed immediately.
• For glucose estimation from urine, add 5ml glacial acetic acid as preservative to inhibit bacterial growth.
ENZYMETIC DETERMINATION

GOD POD METHOD:

PRINCIPLE:

- Glucose + H₂O + O₂ $\xrightarrow{\text{GOD}}$ Gluconic acid + H₂O₂
- 4 Amino Phenazine + Phenol + H₂O₂ $\xrightarrow{\text{POD}}$ Quinonimine – Pink colour compound
- Intensity is determined at on 505 nm filter.
## PROCEDURE

<table>
<thead>
<tr>
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<th>TEST</th>
<th>STAN.</th>
<th>BLANK</th>
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</thead>
<tbody>
<tr>
<td>1) Glucose reagent (ml)</td>
<td>1.0</td>
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<tr>
<td>2) Serum (ml)</td>
<td>0.01</td>
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<td>3) Glucose standard (ml)</td>
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<td>4) Distilled water (ml)</td>
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<td>0.01</td>
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Mix & keep it for incubation at 37°C for 15 min or at room temperature for 30 min.

Measure the intensity of colour at 505 nm filter (Green filter)
Calculation:

Concentration of Substance = 
\[
\frac{\text{O.D. of Test} - \text{O.D. of Blank}}{\text{O.D. of Std.} - \text{O.D. of Blank}} \times \text{Concentration of Std.}
\]

General Parameter:

- Reaction type: End point
- Standard Concentration: 100 mg/dl
- Linearity is up to 500 mg/dl
- If sample value is 500 mg/dl, dilute the sample 1:2 with distilled water & repeat assay
Hexokinase method

PRINCIPLE:

• Glucose + ATP $\rightleftharpoons$ Glucose 6 phosphate + ADP
• Glucose 6 Phosphate + NAD $\rightleftharpoons$ 6-Phosphogluconate + NADH+H⁺
• Conversion of NADH from NAD at 340nm, increase in O.D. is measured at fix interval
• Increase O.D. /min is directly conc. of glucose in the specimen = Delta O.D.
PROCEDURE:

• Pipette 1.0 ml Of Glucose Reagent in Cuvette & Keep It In a Water-bath at 37 °c For 1min(for incubation)

• Add 10 μl of sample mix well & read change in O.D /minute , up to 3 minute

• Repeat steps 1,2 & 3 by using Standard.

CALCULATION:

• Plasma glucose = \frac{\text{Delta O.D./min(test)}}{\text{Delta O.D./min(Std.)}} \times 100
3. GLUCOSE DEHYDROGENASE METHOD

- \text{GLUCOSE} \leftrightarrow \text{D-GLUCONO-δ-LACTONE}
- \text{NAD}^+ \leftrightarrow \text{NADH} + \text{H}^+
4. Orthotoluidine method

- **PRINCIPLE:**
  - Glucose react with orthotoluidin in hot acidic medium to form a Green color complex
  - Color intensity $\alpha$ Conc. Of Glucose
  - Measured in photometer at 620 nm to 660 nm.
  - It can measured other monosaccharide also.
  - It is Non-Specific Method.
  - And Orthotoluidine is carcinogenic, so not utilized nowadays.
5. Folin Vui Method

- Time consuming method
- Non specific method, also measure fructose.
Glucometer

Blood is placed onto a test strip & insert into the glucometer to measure blood sugar levels.

It is only type of dry chemistry
Advantage : Can do from capillary collection method. E.g. Heal Pick, Pinna Pick
Gives result with in second.
Disadvantage : Costly.
Slightly high result than actual.
MEASUREMENT OF GLUCOSE IN URINE

METHOD:
1. Qualitative
2. Quantitative
3. Semi-quantitative

1) QUALITATIVE METHOD:
• It is determination by Benedict test
2) QUANTITATIVE METHOD:
• It Is Determination By Hexokinase & Glucose Dehydrogenase

3) SEMI QUANTITATIVE METHOD:
• It is determination by Glucose Oxidase strip test
• E.g. Urine strip
Benedict's Test

This is a very simple and effective method of the amount of glucose in the urine

❖ Principle:
• Glucose(R-CHO) + 2Cu²⁺ + 2H₂O → Gluconic acid(R-COOH) + Cu₂O + 4H⁺

❖ Procedure:
• 5 ml of Benedict's reagent + 8 to 10 drops of urine Boiling the mixture & cool down it, observe changes colour.
Result & Interpretation on Benedict Test

- Blue - sugar absent;
- Green - 0.5 gm% sugar = +1
- Yellow – 1.0 gm% sugar = +2
- Orange - 1.5 gm% sugar = +3
- Brick red – 2.0 % or more sugar = +4
Significant of Benedict Test

• If blood glucose level cross renal threshold, than it excreted in urine. E.g. in diabetes Mellitus

• If Renal threshold for glucose decrease in renal failure, so in this case also benedict test come positive.

• Each reducing substance gives positive test

• So Following substance can gives false positive test E.g. Vitamin – C, B-Complex vitamin, Salicylic acid
Glucose Oxidase Test

• Paper or plastic strips, called diastix.
• A color-chart is provided with the strips.
• Strip contain dye are O-tolidine, tetramethylbenzidine, potassium iodide, 4-amino phynazome, phenol.
• The dye changes colour on coming in contact with the urine.
• After 30 to 60 seconds the colour of the strip matched with the colours of the provided chart.
Oxidase Strip
• **GLUCOSE ESTIMATION IN CSF**
  
  • CSF is a fluid that flows through and protects the subarachnoid space of the brain and spinal cord.
  
  • It's obtained by lumbar puncture, L 3-L 4
  
  • In CSF, Glucose is estimation by GOD - POD method.
  
  • In CSF Contain
    - 15– 45 mg% Glucose
Clinical interpretation:
An increased CSF glucose level is seen in hyperglycemia.
Decreased CSF glucose in
1. Bacterial Infection
2. Hypoglycemia
CLINICAL SIGNIFICANCE

- Increased glucose: (hyper glycemia)
  - Diabetes mellitus,
  - Hyper thyroidism,
  - Hyper pituitarism,
  - Adrenocortical hyper activity,
- Decreased glucose: (hypo glycemia)
  - Hypo thyroidism,
  - Hypo pituitarism,
  - Hypo adrenalism,
NORMAL RANGE

BLOOD:
- Random Blood Sugar: < 140 mg/dl
- Fasting Blood Sugar: 70 to 110 mg/dl
- Post Parendial Blood Sugar: < 140 mg/dl
- CSF: 40 to 70 mg/dl (1/3 of plasma glucose)
- Urine: Absent