

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, erythosytes & 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.

ENZYMATIC DETERMINATION

GOD POD METHOD:

PRINCIPLE:

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

❖ PROCEDURE

	TEST	STAN.	BLANK
1)Glucose reagent (ml)	1.0	1.0	1.0
2)Serum(ml)	0.01	---	----
3)Glucose standard(ml)	---	0.01	----
4)Distilled water(ml)	---	----	0.01

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)**

B

S1

T

Blood Glucose



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 500mg/dl ,dilute the sample 1:2 with distilled water & repeat assay

➤ Hexokinase method

❖ PRINCIPLE:

- $\text{Glucose} + \text{ATP} \leftrightarrow \text{Glucose 6 phosphate} + \text{ADP}$
- $\text{Glucose 6 Phosphate} + \text{NAD} \leftrightarrow \text{6-Phosphogluconate} + \text{NADH} + \text{H}^+$
- 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

- GLUCOSE \leftrightarrow D-GLUCONO- δ -LACTONE



4.Orthotoluidine method

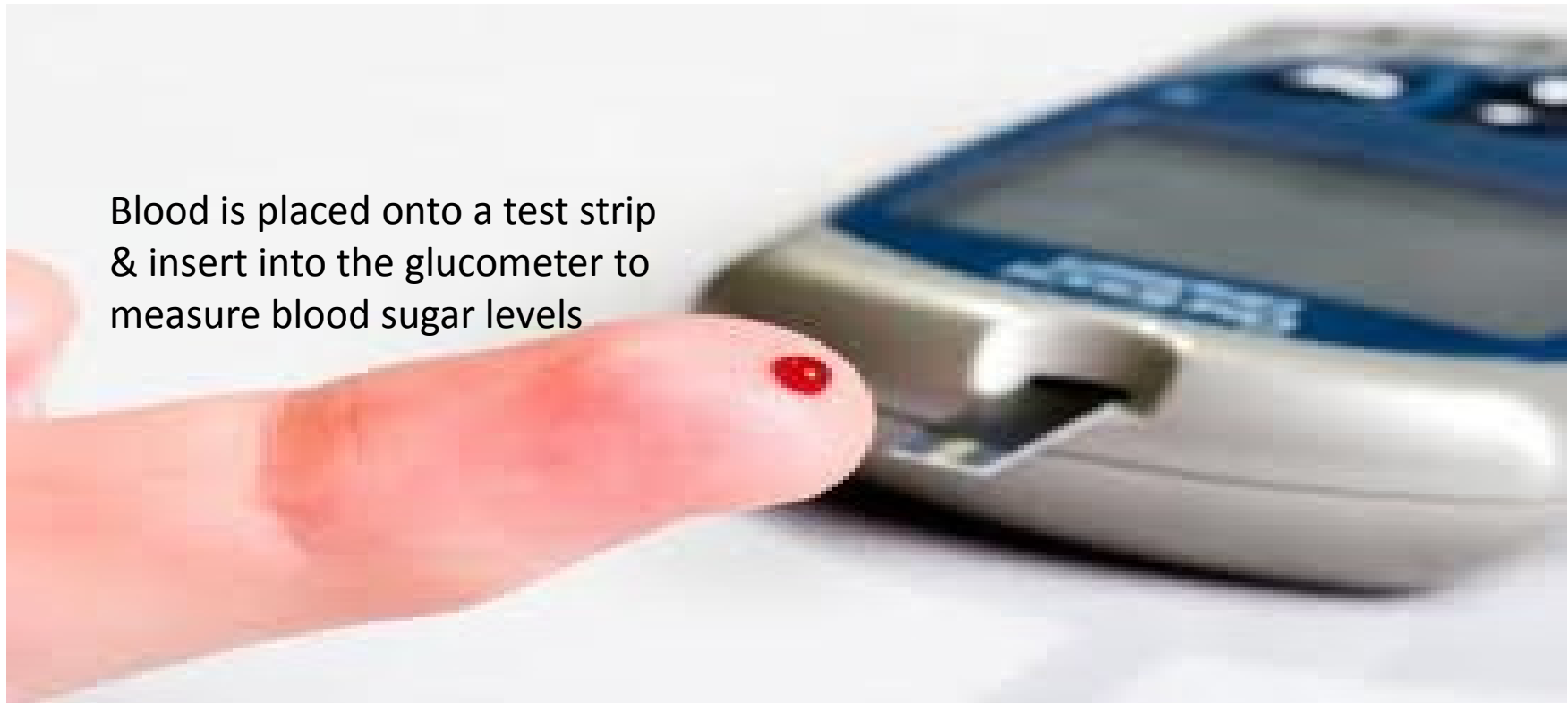
➤ PRINCIPLE:

- Glucose react with orthotoluidin in hot acidic medium to form a Green color complex
- Color intensity \propto 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



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

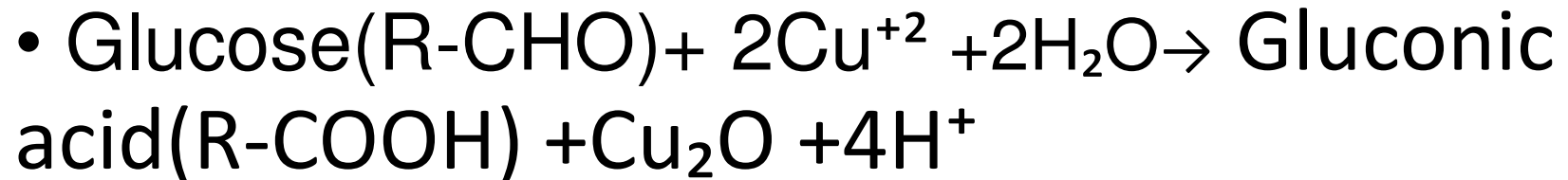
3) SEMI QUANTITATIVE METHOD:

- 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:



❖ 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



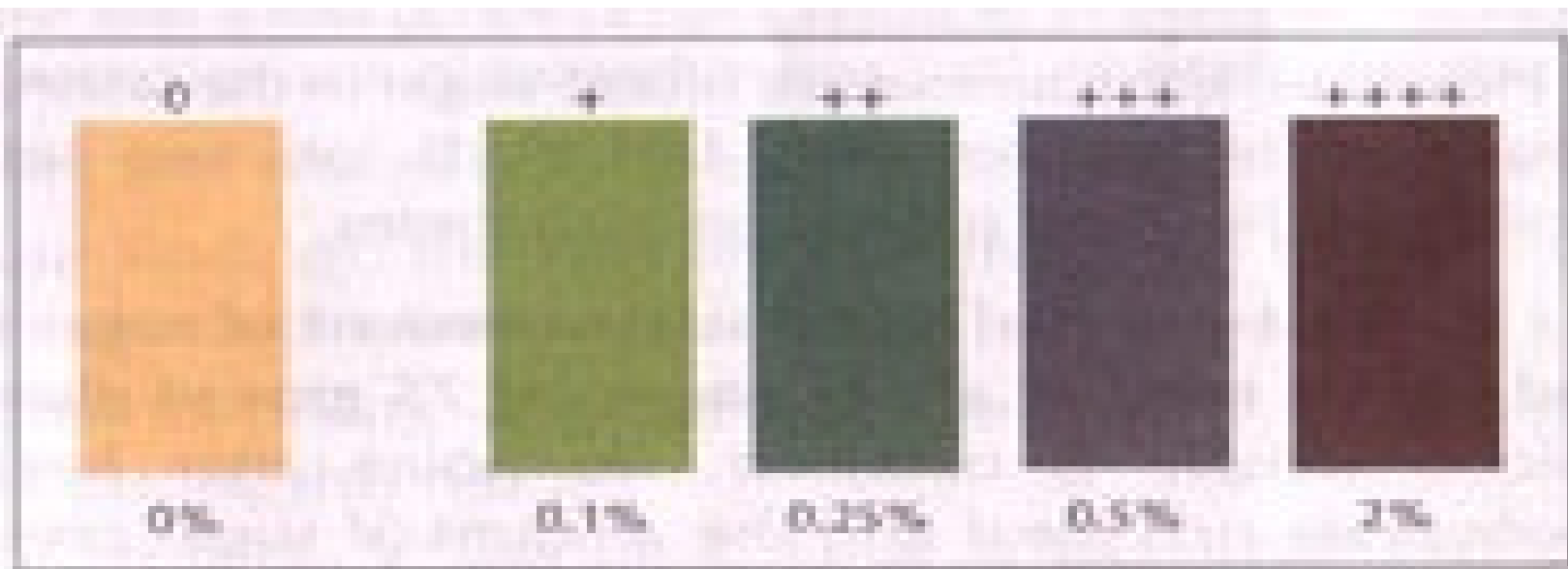
Significant of Benedict Test

- 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 Parential Blood Sugar : <140 mg/dl
- CSF : 40 to 70 mg/dl (1/3 of plasma glucose)
- Urine : Absent



Tag By Adena

Thank You