GLUCOSE

(GOD-POD Method)

Liquid Reagent

INTENDED USE:

This reagent kit is used for *in-vitro* quantitative determination of Glucose in human serum and plasma.

TEST PRINCIPLE:

Glucose present in the plasma is oxidized by the enzyme glucose oxidase (GOD) to gluconic acid with the liberation of hydrogen peroxide, which is converted to water and oxygen by the enzyme peroxidase (POD).

4-aminoantipyrine, an oxygen acceptor, takes up the oxygen and together with phenol forms a pink coloured chromogen which can be measured at 505 nm.

REACTION :

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{GOD}} \text{Gluconic acid} + H_2O_2$$

$$2\,H_2O_2 + 4\,\text{AAP} + \text{Phenol} \xrightarrow{\text{POD}} \text{Red Quinoneimine} + 4\,H_2O$$

KIT CONTENTS:

Reagent 1 : Glucose Enzyme Reagent

Reagent 2 : Glucose Standard (100 mg/dl)

Product Insert : 01 No.

PREPARATION OF THE WORKING REAGENT:

All the reagents are ready to use.

STORAGE AND STABILITY:

All the reagents should be stored in 2-8°C and are stable till the expiry date mentioned in the labels.

SPECIMEN COLLECTION AND STORAGE:

Serum/plasma can be used as sample. Serum should be separated from blood as soon as possible. For plasma: collect venous blood in tubes containing oxalate fluoride.

PRECAUTIONS:

1. Storage conditions as mentioned on the kit to be adhered.
2. Do not freeze or expose the reagents to higher temperature as it may affect the performance of the kit.
3. Before the assy bring all the reagents to room temperature.
4. After use store the kit contents immediately as 2-8°C.
5. Avoid contamination of the reagent during assay process.
6. Use clean glassware free from dust or debris.

PROCEDURE (Automated):

Refer to specific instrument application instructions.

TEST PROCEDURE (Manual): END POINT:

Pipette into clean dry test tubes labeled Blank (B), Standard (S) and Test (T) as follows:

Pipette into clean dry Test Tube	Blank	Standard	Test
Glucose Reagent	1.0 ml	1.0 ml	1.0 ml
Standard	-	10 µl	-
Sample	-	-	10 µl

Mix well and incubate for 10 minutes at 37°C or 20 minutes at CRT (21-25°C).

Read absorbance of Standard (A_S), Test (A_T) and Reagent Blank (A_B) at 505nm or with Green filter (500-540nm).

CALUCATION:

Glucose Conc. in mg/dl =

$$\frac{\text{Abs of } A_T - A_B}{\text{Abs of } A_s - A_B} \times 100 \text{ (Conc. of Standard)}$$

TEST PROCEDURE (Manual): INITIAL RATE KINETIC:

Pipette into test tubes Standard (A_S) and Test (A_T) as follows:

Pipette into Test Tube	Standard	Test
Glucose Reagent	1.0 ml	1.0 ml
Standard	10 µl	-
Sample	-	10 µl

Mix and aspirate. After 1 minute incubation, measure the change in absorbance (ΔAbs) for one minute for standard and test. Use this (ΔAbs) for calculations.

CALCULATIONS:

Glucose Conc. in mg/dl =

$$\frac{\Delta \text{ Abs of Test}}{\Delta \text{ Abs of Std.}} \times 100 \text{ (Concentration of Standard)}$$

NORMAL VALUES*:

Fasting : 70 - 100 mg/dl

Post-prandial : Upto 140 mg/dl

*It is recommended that each laboratory should establish its own normal range.

PERFORMANCE:

1. **LINEARITY :** 500 mg/dl
2. **Comparison :**

   A comparison between Glucose Liquid Reagent (y) and a commercially available test(x) using 55 samples gave the following results: y = 1.117 x -2.510, r = 0.99.

3. **Precision :**

	Within Run			Run to Run		
	Mean	S.D.	C.V.%	Mean	S.D.	C.V.%
Low	95.0	0.4	0.4	95.0	1.5	1.5
High	240.0	1.3	0.9	246.0	2.5	1.2

4. **Specificity:** No interference up to Hemoglobin 1 gm/l & Bilirubin 30 mg/dl.

CLINICAL SIGNIFICANCE:

Glucose is are reducing monosaccharide that serves as the principal fuel for all the tissues. It enters the cell through the influence of insulin and undergoes a series of chemical reactions to produce energy.

Lack of insulin or resistance to its action at the cellular level causes diabetes. Therefore, in diabetes mellitus the blood glucose levels are very high. Some patients with very high blood glucose levels may develop metabolic acidosis and ketosis caused by the increased fat metabolism, the alternate source for energy. Hyperglycemia is also noted in gestational diabetes of pregnancy and may be found in pancreatic disease, pituitary and adrenal disorders.

A decreased level of blood glucose, hypoglycemia is often associated with starvation, hyper insulinaemia and in those who are taking high insulin dose for therapy.

AUTOMATED APPLICATIONS:

Glucose Liquid Reagent reagents can be used with Hitachi 700 series, RA 50, 1000XT, Express 550, Syncron CX4, LISA 200, BTR 810/820/830, Erbachem-5, Ranlab etc. Application sheets for use on specific semiautomatic/batch analyzers are available on request.

Input parameters for semi-auto/auto analyzers are given below:

END POINT METHOD

INPUT PARAMETERS	VALUES
Type of reaction	Endpoint
Wavelength	505 nm.
Incubation time	10 minutes
Standard concentration	100 mg/dl
Units	mg/dl
Temperature	37°C
Upper Normalvalue	140 mg/dl
Lower Normalvalue	70 mg/dl
Linearity	500 mg/dl
Reagent volume	1.0 ml
Sample/Standard volume	10 µl

KINETIC METHOD

INPUT PARAMETERS	VALUES
Type of reaction	Initial rate kinetic
Wavelength	505 nm.
Incubation time	60 seconds
Reading time	60 seconds
Standard concentration	100 mg/dl
Units	mg/dl
Temperature	37°C
Upper Normalvalue	140 mg/dl
Lower Normalvalue	70 mg/dl
Linearity	500 mg/dl
Reagent volume	1.0 ml
Sample/Standard volume	10 µl

QUALITY CONTROL:

For accuracy, it is necessary to run known serum controls with each assay.

REFERENCES:

1. Trinder, P. (1969). Annals of Clin. Biochem. 6:24-27.
2. Barham Dand Trinder P. (1972). Analyst 97:142-145.
3. Bergmayer, H.V. (1974) Method of Enzymatic Analysis. P.1196.

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