GLUCOSE

GOD-POD Method

Intended Use:
Glucose Test reagent/kit is a medical device intended for estimation of Glucose in serum/plasma/blood/blood fluids.

Clinical Significance
Glucose is the major carbohydrate present in blood. Its oxidation in the cells is the source of energy for the body.
Increased levels of glucose are found in diabetes mellitus, hyperparathyroidism, pancreatitis, renal failure.
Decreased levels are found in insulinoma, hypothyroidism, hypopituitarism and extensive liver disease.

Principle

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

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

GOD – Glucose Oxidase 4AAP – 4 Amino Antipyrine
POD – Peroxidase
Intensity of the colour formed is directly proportional to the amount of glucose present in the sample.

Reagent Composition:

GOD	≥ 13 KU/L	4-AAP	≥ 1 mmol/L
POD	≥ 2 KU/L	Buffer	≥ 180 mmol/L
Phenol	≥ 6 mmol/L	Stabilizers and Activator	

Working Reagent Preparation
Reagent is ready to use.

Stability and Storage
Store at 2-8ºC
All the kit contents are stable until the expiry date stated on thelabel. Do not use reagents beyond the expiration date.
Store the vials tightly closed protected from light and prevent contaminations during the use.
Upon storage the Glucose reagent may develop a slight pink colour. This does not affect the performance of the test.

Discard if signs of deterioration appear:
- Presence of particles and turbidity.

Materials required
- Photometer or spectrophotometer with a thermostat cell compartment set at 25/30/37ºC, capable of reading at 505 (500-540) nm.
- Stopwatch, strip-chart recorder or printer.
- Cuvettes with 1-cm path length
- Pipettes to measure reagent and samples.

Sample and Stability
Serum or plasma
Serum or heparinized plasma free of hemolysis.
Glucose is reported to be stable in the sample for 7 days when stored at 2-8^{0}C.

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

Addition Sequence	Blank	Std	Test
R1 Glucose Reagent	1000µl	1000µl	1000µl
Standard (S)	-	10µl	-
Sample	-	-	10µl

Mix well and incubate at 37^{0}C for 10 min or at R.T(<30^{0}C) for 15 min. Measure absorbance of the Standard (Abs.S) and Test Sample (Abs.T) against Blank

Calculations:

$$\text{Glucose in mg/dl} = \frac{\text{Abs.T}}{\text{Abs.S}} \times 100$$

Kinetic Procedure:
Bring the Glucose reagent to R.T. before use, pipette into test tubes labeled Standard (S) and Test (T) as follows:

Addition Sequence	Std	Test
R1 Glucose Reagent	1000µl	1000µl
Standard (S)	10µl	-
Specimen	-	10µl

Mix well and read absorbance of S and T at 37^{0}C against distilled water at 505 nm (500-540) kinetically as follows:
Initial absorbance Ao = Exactly after 20 sec.
Final absorbance A1 = Exactly 40 sec, after Ao
Determine Δ Abs for S and T.
Δ Abs S = Abs S_1 – Abs So
Δ Abs T = Abs T_1 – Abs To

Calculations:

$$\text{Glucose in mg/dl} = \frac{\text{Abs.T}}{\text{Abs.S}} \times 100$$

Quality Control
To ensure adequate quality control (QC), each run should include aset of controls (normal and abnormal) with assayed values handledas unknowns.
If the values are found outside of the defined range, check the instrument, reagents and procedure.
Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable results

Linearity
This procedure is linear upto 600 mg/dl. If values exceed this limit, dilute the serum with normal saline (NaCl 0.9%) and repeat the assay, calculate the value using the proper dilution factor.

Reference Values

Serum/Plasma (Fasting)	:	70-110 mg/dl
Post Prandial	:	Upto 150 mg/dl
CSF	:	50-80 mg/dl

It is recommended that each laboratory establish its own normal range representing its patient population.

General System Parameters

Mode	End Point
Reaction	Increasing
Wavelength	505 (500-540) nm
Blank with	Reagent
Sample Volume	10µl
Reagent Volume	1000µl
Std Conc.	100 mg/dl
Incub. Temp.	37^{0}C
Incub. Time	10 min.
Delay Time	5 sec
Normal Range	70 – 110 mg/dl
Linearity	Up to 600mg/dl
Unit	mg/dl

Notes
1. To avoid glycolysis the serum should be separated from the clot as soon as possible, and plasma should be collected in an EDTA+fluoride bulb (0.5 mg + 1 mg per ml of blood).
2. Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.

Reference
1. Trinder, P., (1969) Ann. Clin. Biochem. 6:24

For *in vitro* Diagnostic use only.

Belongs to human life

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