GLUCOSE LYPHO

(GOD-POD Method)

Intended Use

Glucose is a reagent kit used for the determination of true glucose in serum or plasma, based on enzymatic method; using glucose oxidase and peroxidase enzymes.

Principle

Glucose oxidase (GOD) converts glucose into gluconic acid and Hydrogen peroxide (H_2O_2). Hydrogen peroxide, in presence of peroxidase (POD), oxidatively couples with 4-aminoantipyrine and phenol to produce red quinoneimine dye. This dye has absorbance maximum at 505 nm (500 - 550 nm). The intensity of the colored complex is directly proportional to the concentration of glucose in specimen.

$$\beta\text{-D Glucose} + O_2 + H_2O \xrightarrow{GOD} \text{Gluconic Acid} + H_2O_2$$

$$H_2O_2 + \text{4-aminoantipyrine} + \text{phenol} \xrightarrow{POD} \text{Red dye} + H_2O$$

Reagents provided

1. R1 - Diluent
2. R2 - Glucose enzyme Vial
3. Standard - Glucose (100 mg/dl).

Working Reagent Preparation

Reconstitute enzyme & Diluent as per instruction indicated on individual bottle label to prepare working solution. Mix by gentle swirling or inversion. DO NOT SHAKE VIGOROUSLY.

Reagent storage and stability

Enzyme and Standard should be stored at 2° - 8°C. Diluent should be stored below 30°C and away from direct light. The working solution is stable for 60 days when stored at 2° - 8°C (Do not freeze). The working solution should be stored in the dark bottle(Working solution container) provide. This is critical because the reagent is light sensitive(auto oxidation of chromogen system by light and air), it should therefore be kept away from direct light.

Specimen collection and preservation

Blood should be collected in a clean dry container. Serum or plasma should be separated from the cells at the earliest possible (within 30 minutes), as the rate of glycolysis is approximately 7 mg% per hour at room temperature (25° - 30°C).

For plasma separation following anticoagulants may be used.

- EDTA 2 mg/ml of blood
- CITRATE 6 mg/ml of blood
- HEPARINE 200 IU/ml of blood
- OXALATE 3 mg/ml of blood
- SODIUM FLUORIDE 10 mg/ml of blood

Sodium fluoride is preferred anticoagulant due to its antiglycolytic activity. Higher concentration of sodium fluoride i.e. more than 10 mg/ml of blood should be avoided as it may inhibit the colour development.

Glucose is stable for 24 hours in neatly separated plasma and serum at 2° - 8°C. If the estimation is not possible within 24 hours then the specimen should be preserved at -20°C and should be used within 30 days.

Assay guidelines for Analyzers

Reaction Type	End Point with Standard
Reaction slope	Increasing
Incubation time	15 min. at 37°C / 30 min. at RT (25°-30°C).
Wave length	505 nm (490 - 530 nm)
Blank	Reagent Blank
Blank absorbance limit	< 0. 300 Abs. against distilled water blank.
Sample Volume	10 µl (0.01 ml)
Reagent Volume	1000 µl (1.0 ml)
Glucose Standard Concentration	100 mg/dl
Factor Calculation	100 mg/dl ÷ Absorbance of Standard.
Low normal	70 mg/dl
High normal	140 mg/dl
Linearity	Up to 450 mg/dl

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Assay guidelines for Manual Procedure

Bring the working reagent and standard to room temperature before performing the assay.

Reagents	Blank	Standard	Sample
Reagent	1000 µl (1.0 ml)	1000 µl (1.0 ml)	1000 µl (1.0 ml)
Standard	-	10 µl (0.01 ml)	-
Sample	-	-	10 µl (0.01 ml)

1. Mix thoroughly and incubate at 37°C for 15 minutes or 30 minutes at room temperature (25° - 30°C).
2. Read the absorbance against reagent blank at 505 nm (490 - 530nm).
3. The final colour is stable for 2 hours if not exposed to direct light.

Calculation

Glucose conc. in sample (mg/dl) = $\frac{\text{Sample OD}}{\text{Std. OD.}}$ x Con. of Std.

Normal Range

Guidance value for Fasting serum / Plasma : 70 - 110 mg/dl
Guidance value for Post Prandial / Random : Up to 140 mg/dl
Note: Expected range varies from population to population and each laboratory should establish its own normal range.

Limitation

Reaction is linear up to 450 mg/dl. For higher values, dilute the sample with normal saline and perform the assay. Multiply the final result by dilution factor to get the real value.
Discard the reagent if the absorbance exceeds 0.300 against distilled water as blank at 505 nm.

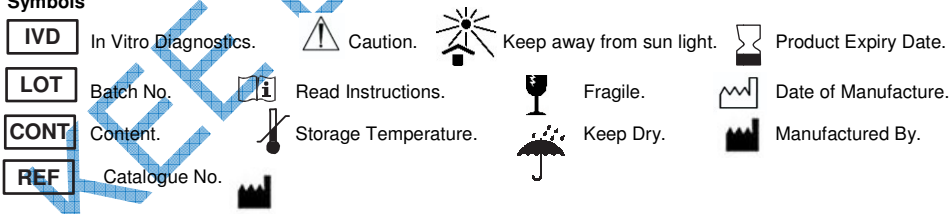
Quality Control

To ensure adequate quality control measures, it is recommended that each batch should include a normal and an abnormal commercial reference control serum. It should be realized that the use of quality control material checks both instrument and reagent functions together. Factors which might affect the performance of this test include proper instrument function, temperature control, cleanliness of glassware and accuracy of pipetting.

References

1. Trinder P. Clin. Biochem. 6, 24 (1969).
2. Bergmayer H. V., Methods of enzymatic Analysis", A. P., N. Y. (1974). Page 1196.
3. Young, D.S., Pestaner, L.C., Gibberman, B., *Clin. Chem.* 21, 1D (1975).

Symbols



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