GLUCOSE
GOD-POD Method

DIAGNOCHEM

DIAGNO CARE

CLINICAL SIGNIFICANCE :

Determination of glucose concentration is important in the diagnosis and treatment disorders of carbohydrate metabolism. 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.

TEST PRINCIPLE :

Determination of glucose after enzymatic oxidation by glucose oxidase (Trinder's reaction) [3].

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

$$2\,H_2O_2 + \text{4-Aminoantipyrine} + \text{Phenol} \xrightarrow{\text{POD}} \text{Quinoneimine} + 4\,H_2O$$

Glucose oxidase (GOD) converts the sample glucose into gluconate. The hydrogen peroxide (H_2O_2) produced in the reaction is degraded by peroxidase (POD) and gives a colored product Phenol and 4-Aminoantipyrine which is measurable using Trinder indicator reaction at 505 nm. The increase in absorbance correlates with the glucose concentration of the sample.

REAGENTS COMPOSITION :

Reagent R1

Phosphate Buffer > 150 mmol/L
Phenol > 6 mmol/L
4-Aminoantipyrin > 1 mmol/L
Glucoseoxidase (GOD) > 13 KU/L
Peroxidase(POD) > 2 KU/L
Stabiliser and Activator

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2 - 8 ºC, and contaminations prevented during their use. Do not use reagents over the expiration date. Once opened the reagent is stable for at least 3 months at 2 – 8 °C and 1 month on-board the auto analyzer at approximately 10°C.

KIT CONTENTS :

CODE No. : GL01
Pack size : (5x100ml)
Reagent-1 (R1)
Glucose Reagent 5 x 100 ml
Glucose Standard 1 x 2 ml
(Conc : 100mg/dl)

MATERIALS REQUIRED BUT NOT PROVIDED :

Glucose Control (Use of assayed QC sera is recommended to validate test result).

SAMPLES :

Serum, heparinized plasma, Fluoride plasma, CSF

Serum or plasma must be separated within 30 mints of collection. Separated Plasma or Serum is stable for 24 hours at +2 to +8°C.

INTERFERENCES

Gross haemolysis, sample interfere with the results. Samples with elevated bilirubin (> 20mg/dl), Hemoglobin (>100 mg/dl), Intralipid (> 1000 mg/dl) and Ascorbic Acid (>20mg/dl) may have a slight effect on accuracy.

ASSAY CONDITIONS:

Wavelength : ..... 505 nm (490 - 546 nm)
Cuvette: ..... 1 cm light path
Constant temperature 37ºC
Reaction ..... End Point
Standard Conc..... 100
Linearity..... 600 mg/dL
Unit..... mg/dl
Slope of Reaction ..... Increasing
Blanking..... Reagent

PROCEDURE :

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

	B	S	T
Glucose Reagent (R1)	1.0 ml	1.0 ml	1.0 ml
Glucose Calibrator		10 µl	
Specimen			10 µl

Mix and incubate for 10 mint at 37ºC or 15 mint. at RT (25ºC - 30ºC) Read absorbance of Calibrator (S) and Test (T) against Blank (B) with 505 nm. The final color is stable for 1 hour at R.T.

CALCULATIONS :

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

REFERENCE RANGE :

Serum/Plasma Fasting : 65 - 110 mg/dL
Serum/Plasma PP : < 140 mg/dL
CSF : 50 - 80 mg/dL

The above reference range is guideline and all the laboratories must establish their own normal reference range. Final diagnosis should be made with correlation of clinical factors.

PRECAUTIONS :

1. Storage conditions as mentioned on the kit to be adhered.

2. Use clean glassware and microtips while pipetting Calibrator. Replug properly Calibrator vial after use

3. Avoid contamination of the reagent during the assay process.

4. Before the assay begins, bring all the reagents to room temperature.

5. If a larger volume of reagent is required for the absorbance reading, requisite volume can be taken in multiples, keeping the same ratio of reagent to specimen/standard.

6. Do not freeze or expose the reagents to high temperature and protect from direct sunlight as it will affect the performance of the kit.

7. Programmes for specific autoanalysers are available on request.

8. For accuracy of results, the assay procedure, reagent preparation and storage has to be meticulously followed.

9. As with all the diagnostic procedures, the physician should evaluate data obtained by the use of this kit in light of other clinical information.

LINEARITY AND DETECTION LIMIT :

The assay is linear up to Glucose concentration of 5 - 600mg/dl The results of the performance characteristics depend on the analyzer used. If the results obtained were greater than linearity limit, dilute the sample 1 : 4 with Normal S aline and multiply the result by 4.

BIBLIOGRAPHY :

1. Thomas L. Clinical Laboratory Diagnostics. 1st ed.Frankfurt: TH-Books Verlagsgesellschaft; 1998. p.131-7.
2. Sacks DB. Carbohydrates. In: Burtis CA, AshwoodER, editors. Tietz Textbook of Clinical Chemistry. 3rded. Philadelphia: W.B Saunders Company; 1999. p. 750-808.
3. Barham D, Trinder P. An improved color reagent for the determination of blood glucose by the oxidase system. Analyst 1972;97:142-5.

Manufactured by : **DIATEK Healthcare Pvt. Ltd.**
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Medical Device Insert / Kit Insert / Instruction for use (IFU) : GLU/KI/DC/01 : 01/09/2022 Rev:00

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