

GLUCOSE(GOD-POD)

INTENDED USE

Bioline Glucose reagent is used for the quantitative determination of glucose in serum.

CLINICAL SIGNIFICANCE

Glucose is the major carbohydrate present in the peripheral blood. The oxidation of glucose is the major source of cellular energy in the body. Glucose determinations are run primarily to aid in the diagnosis and treatment of diabetes mellitus. Elevated levels of glucose levels maybe associated with pancreatitis, pituitary or thyroid dysfunction, renal failure and liver disease, whereas low glucose levels may be associated with insulinoma, hypopituitaryism, neoplasms, or insulin induced hypoglycemia.

METHOD AND PRINCIPLE

Early enzymatic methods for glucose determination involved glucoseoxidase to catalyze the oxidation of glucose. Keston modified this method in the early 1950's using glucose oxidase/peroxidase enzyme system and O-dianisidine chromogen system. Since then, various alternative chromogen systems have been proposed. The trinder method replaces carcinogenico-dianisidine with phenol plus 4-aminoantipyrine. This method is less influenced by interfering substances and does not suffer from the many drawbacks of earlier methods. The enzymatic reaction sequence employed in the assay of glucose is as follows:

D-Glucose +H₂O+O₂ ----- H₂O₂+D-Gluconic acid

H₂O₂+4AAP+Phenol----- Quinonemine +H₂O

D-Glucose is oxidized by glucose oxidase to produce D-gluconic acid and hydrogen peroxide. The hydrogen peroxide is then oxidatively coupled with 4-aminoantipyrine and phenol in the presence of peroxidase to yield a red quinoneimine dye. The amount of colored complex formed is proportional to glucose concentration and can be photometrically measured

REAGENT COMPOSITION

1. Glucose (Liquid) Reagent :Glucose Oxidase15IU/ml, Peroxidase (horseradish) 1.2 IU/ml. 4-Aminoantipyrine0.2mM, Phenol 4mM, non-reactive ingredients and preservatives.
2. Glucose Standard: 100 mg/dl D-glucose in aqueous solution.

WARNINGS AND PRECAUTIONS

1. For *in vitro* diagnostic use.
CAUTION: *In vitro* diagnostic reagents may be hazardous. Handle in accordance with good laboratory procedures which dictate avoiding ingestion, and eye or skin contact.
2. Specimens should be considered infectious and handled appropriately. Use distilled or deionized water where indicated.

REAGENT PREPARATION

Reagent and standard are ready for use

REAGENT STORAGE AND STABILITY

Both liquid reagent and standard should be stored at 2 - 8°C. The reagent may be used until the expiration date indicated on the package label.

REAGENT DETERIORATION

The reagent should be discarded if:

1. Turbidity has occurred; turbidity may be a sign of contamination.
2. The reagent fails to meet linearity claims or fails to recover

control values in the stated range.

SPECIMEN COLLECTION AND STABILITY

1. Test specimens should be serum and free from hemolysis.
2. Plasma should be collected in Fluoride or heparin/iodoacetate or any other inhibitor of glycolysis.
3. Serum must be separated from the clot promptly since the rate of glucose decrease is approximately 7% per hour in whole blood.
4. Glucose in serum or plasma is stable for twenty-four (24) hours when stored at 2 - 8° C.

INTERFERENCES

Grossly lipemic or icteric sera will cause false glucose values and require the use of a serum blank. Add 0.02 ml (20 µl) of patient sera to 3.0 ml distilled water and read against a water blank. Subtract this absorbance from the patient test absorbance to correct for the lipemia or icterus. Young, et al .give a comprehensive review of drug interferences.

ASSAY PROCEDURE FOR SEMIAUTO ANALYZER

Wavelength : 505 nm

Temperature : 37°C

	Blank	Standard	Sample
Reagent	1mL	1mL	1mL
Distilled water	10µL	-	-
Standard	-	10µL	-
Sample	-	-	10µL

Mix and measure the optical density (OD) of standard and sample against reagent blank after 10 minutes of incubation. The final color is stable for 30minutes.

CALCULATIONS

(A= Absorbance)

$$\frac{A(\text{sample})}{A(\text{standard})} \times \text{Concentration of standard} = \text{Concentration of sample}(\text{mg/dl})$$

Example: A (sample) = 0.37, A (standard) = 0.28
Concentration of standard = 100 mg/dl

$$\text{Sample concentration} = \frac{0.37 \times 100}{0.28} = 132 \text{ mg/dl}$$

CALIBRATION

The procedures are calibrated with the standard solution which is included with each series of tests. Its absorbance is used to calculate results. It is recommended to establish a linearity curve up to 500mg/dl with other available commercial standard solutions to verify the performance of the instruments and reagents.

LIMITATIONS

The reagent is linear to 500 mg/dl glucose. Samples that have glucose values greater than 500mg/dl should be diluted with water 1:1, reassayed and the results multiplied by 2.

QUALITY CONTROL

It is recommended that high and low values of glucose controls be included in each set of assays. Commercially available control material with established glucose values may be used for quality control. The assigned value of the control material must be confirmed by the chosen application. Failure to obtain the proper range of values in the assay of control material may indicate either reagent deterioration, instrument malfunction or procedural errors.

EXPECTED VALUES

Fasting Serum Glucose 70-105 mg/dl

Postprandial serum Glucose 90-140 mg/dl

PERFORMANCE CHARACTERISTICS

1. Linearity: 500mg/dl.
2. Sensitivity: An absorbance change of 0.001 at 505nm corresponds to 0.5 mg/dl under the stated condition of this assay system.
3. Comparison: A comparison between this reagent and competitor produced a regression equation of: $y = 1.00x + 2.54$ (N= 64) with a coefficient of correlation of 0.99.

4. Precision:

Within Run

	Sample 1	Sample 2
Mean	83	313
SD	4.7	18.8
CV%	4.2	3.8

Between Run

	Sample 1	Sample 2
Mean	83	313
SD	4.5	19.8
CV%	5.6	6.0

GENERAL TECHNICAL PARAMETER

Mode	End Point
Wavelength (Filter)	505 nm
Reaction Direction	Increasing
Reagent Blank	Yes
Sample Vol.	10 µL
Reagent Vol.	1000 µL
Incubation Time	10 min
Reagent Blank Abs.(Max)	NMT 0.300 Abs
Calibration Method	1- Point
Standard (Conc.)	100mg/dL
Linearity	500mg/dL
Decimal Places	1
Temp.	37°C
Unit	mg/dL
Ref. Low (Male / Female)	70mg/dL
Ref. High (Male / Female)	105mg/dL

REFERENCES

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