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GLUCOSE OXIDASE REAGENT SET (PHENOL FREE)

GLUCOSE (OXIDASE) REAGENT SET (PHENOL FREE)
For the quantitative determination of total glucose in serum.

INTRODUCTION
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 glucose levels may be 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.[1,2]

Early enzymatic methods for glucose determination involved glucose oxidase to catalyze the oxidation of glucose. Keston modified this method in the early 1950's using a glucose oxidase/peroxidase enzyme system and o-dianisidine chromogen system.[3] Since then various alternative chromogen systems have been proposed. The Trinder method replaces carcinogenic o-dianisidine with phenol plus 4aminoantipyrine.[4] This method is less influenced by interfering substances and does not suffer from the many drawbacks of earlier methods.

PRINCIPLE
The enzymatic reaction sequence employed in the assay of glucose is as follows:

$$\beta\text{-D-Glucose} + H_20 + 0_2 \xrightarrow{\text{Glucose Oxidase}} H_20_2 + \text{D-Gluconic Acid}$$

$$H_20_2 + \text{4-Aminoantipyrine} + \text{p-HBS} \xrightarrow{\text{Peroxidase}} \text{Iminoquinone} + H_20$$

β-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 substitute, p-HBS, 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
When reconstituted as directed, the reagent for Glucose contains the following:

1. Glucose reagent :(Concentrations refer to the reconstituted reagent) Glucose Oxidase 15µl/ml, Peroxidase (horseradish) 1.2µl/ml. Mutarotase 4.0 µl/ml. 4-Aminoantipyrine 0.38 mM.p-Hydroxybenzene sulfonate 10 mM, and non-reactive ingredients.
2. Glucose standard: (100mg/dl β-D-glucose).

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.
3. Use distilled or deionized water where indicated.

STORAGE AND STABILITY
Both dry reagent and standard should be stored at 2 - 8° C prior to reconstitution. The reagent may be used until the expiration date indicated on the package label. The reconstituted reagent should be stored in an AMBER container at 2 - 8° C and is stable for thirty (30) days when stored as directed. The reagent should be clear and colorless.

REAGENT DETERIORATION
The reagent should be discarded if:
1. Turbidity has occurred; turbidity may be a sign of contamination.
2. Moisture has penetrated the vial and caking has occurred.
3. The reagent fails to meet linearity claims or fails to recover control values in the stated range.

SPECIMEN COLLECTION
1. Test specimens should be serum and free from hemolysis.
2. Plasma containing citrate, EDTA, heparin or oxalate as an anticoagulant may not be used.
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 2 - 8° C.

INTERFERING SUBSTANCES
Grossly lipemic or icteric sera will cause false glucose values and require the use of a serum blank. Add 0.02ml (20µl) 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 inteferences.[5]

MATERIALS REQUIRED BUT NOT PROVIDED
1. Pipettes to accurately measure required volumes.
2. Test tubes/rack.
3. Timer.
4. 37° C heating block or water bath.
5. Spectrophotometer capable of accurately measuring absorbances at 500nm.

GENERAL INSTRUCTIONS
The reagent for Glucose is intended for use either as an automated procedure on chemistry instruments or as a manual procedure on a suitable spectrophotometer.

PROCEDURE (AUTOMATED)
Consult the appropriate instrument application guide available from us.

PROCEDURE (MANUAL)
1. Prepare reagent according to instructions.
2. Label test tubes: blank, standard, control, patient, etc.
3. Pipette 1.0 ml of working reagent to all tubes and place in 37°C heating bath for at least five (5) minutes.
4. Add 0.01ml (10µl) of sample to respective tubes, mix and incubate at 37°C for exactly ten (10) minutes.

5. After incubation, zero spectrophotometer with the reagent blank. Read and record the absorbances of all tubes at 500nm (Wavelength range:500-520). Final color is stable for at least thirty (30) minutes.

* USE MULTI PURPOSE CALIBRATOR TO REPLACE STANDARD.

NOTE:

If the spectrophotometer being used requires a final volume greater than 1.5ml for accurate reading, use 0.02ml (20µl) of sample to 3.0ml of reagent. Perform the test as described above.

PROCEDURAL LIMITATIONS

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

CALCULATIONS

(A = Absorbance)

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

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

$$\frac{0.42}{0.28} \times 100 = 132\ mg/dl$$

SI UNITS: To obtain results in SI units (mmol/L), multiply your result in mg/dl by ten (10) to convert dl to liter and divide the value by 180, the molecular weight of glucose.

$$mg/dl \times \frac{10}{180} = mg/dl \times 0.0556$$

Example: 132mg/dl x 0.0556 = 7.34mmol/L

QUALITY CONTROL

It is recommended that 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

70-105 mg/dl[6]

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

PERFORMANCE CHARACTERISTICS

1. Linearity: 500mg/dl.
2. Comparison: A comparison between this procedure and one utilizing phenol free produced a regression equation of y = 1.02X + 3.1 with a coefficient of correlation of 0.99.
3. Precision:

	Within Run	
Mean (mg/dl)	S.D.	C.V.
87	4.2	4.8%
282	5.4	1.9%
	Run to Run	
Mean (mg/dl)	S.D.	C.V.
85	3.7	4.3%
287	9.6	3.3%

REFERENCES

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4. Trinder, P., "Determination of Blood Glucose Using 4-Aminophenazone." *.J. Clin. Path*., 22:246 (1959).
5. Tietz, N.W., *Fundamentals of Clin. Chem*., Philadelphia, W.B. Saunders (1970).

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