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GLUCOSE (LIQUID) REAGENT

INTENDED USE
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 disfunction, 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 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 4-aminoantipyrine.[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:

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

$$H_2O_2 + \text{4-Aminoantipyrine} + \text{p-HBS} \xrightarrow{\text{Peroxidase}} \text{Iminoquinone} + H_2O$$

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
1. Glucose (Liquid) Reagent: Glucose Oxidase 15 IU/ml, Peroxidase (horseradish) 1.2 IU/ml. 4-Aminoantipyrene 0.2mM, p-HBS 4mM, non-reactive ingredients and preservatives.
2. Glucose Standard: 100 mg/dl β-D-glucose in aqueous solution.

REAGENT PREPARATION
All reagents are ready for use.

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.

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
1. Test specimens should be serum and free from hemolysis.
2. Serum must be separated from the clot promptly since the rate of glucose decrease is approximately 7% per hour in whole blood.[5]
3. Glucose in serum or plasma is stable for twenty-four (24) hours whenstored at 2 - 8° C.[5]

INTERFERING SUBSTANCES
Grossly lipemic or iceteric sera will cause false glucose values and require the use of a serum blank.[5] 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.[6]

MATERIALS PROVIDED
1. Glucose (Liquid) Reagent
2. Glucose Standard

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 500 nm

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

AUTOMATED PROCEDURE
Please refer to appropriate application manual available.

MANUAL PROCEDURE
1. Label tubes: blank, standard, control, patient, etc.
2. Pipette 1.5 ml of working reagent to all tubes and place in a 37°C heating bath for at least five (5) minutes.
3. Add 0.01 ml (10 µl) of sample to respective tubes, mix and incubate at 37°C for exactly ten (10) minutes.
4. After incubation, zero spectrophotometer with the reagent blank. Read and record the absorbances of all tubes at 500 nm (Wavelength range: 500 - 520 nm).

** TC MULTI-PURPOSE CALIBRATOR MAY BE USED TO REPLACE STANDARD.*

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

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 500 mg/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 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 = 100 mg/dl

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

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.

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

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

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

70 - 105 mg/dl[5]

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

PERFORMANCE CHARACTERISTICS

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

	Within Run	
Mean (mg/dl)	S.D.	C.V. (%)
83	4.7	5.6
313	18.8	6.0

	Run-to-Run	
Mean (mg/dl)	S.D.	C.V. (%)
83	7.0	8.4
285	24.0	8.5

REFERENCES

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4. Trinder, P., "Determination of blood glucose using 4-aminophenazone." *J. Clin. Path.* 22:246 (1969).
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