Glucose LiquiColor® Test
Procedure No. BD184

For the Quantitative Enzymatic- Colorimetric Determination of Glucose in Serum, Plasma or CSF

Summary and Principle

chemical
procedures employed in clinical laboratory medicine.1 Glucose oxidase methodology was introduced by Keilin and Hartree2 in 1948. Keston3 later reported use of the combined glucose oxidase-peroxidase reagent, followed by the Teller4 addition of a chromogenic reagent to Keston's procedure. The Stanbio single reagent glucose method is based on a technique described by Trinder et al.5
Glucose is oxidized in the presence of glucose oxidase (GO). The hydrogen peroxide formed reacts, under the influence of peroxidase (PO), with phenol and 4-aminoantipyrine to form a redviolet quinone complex. The intensity of the color is proportional to glucose concentration.
Glucose + O_2 + H_2O –(GO)–>Gluconic Acid + H_2O_2
H_2O_2 + 4-aminoantipyrine + Phenol –(PO)–> Quinone Complex

Reagents

Glucose LiquiColor® Reagent, BD184a

Contains:	4-aminoantipyrine	0.2 mmol/L
	Glucose Oxidase	15.0 U/mL
	Peroxidase	1.2 U/mL
	Phenol	4.0mmol/L
	Non reactive ingredients and preservatives	

Glucose Standard, (100 mg/dL), #BD185b
Glucose in aqueous benzoic acid.

Precautions: For In Vitro Diagnostic Use. Dispose of reagents in accordance with local requirements.

Reagent Preparation: Reagent and standard are ready-to-use.

Reagent Storage and Stability: Reagent is stable, when stored at 2-8°C until expiration date on the label. Once opened, contamination must be avoided. Measurement is not influenced by reagent color changes as long as absorbance of the reagent is< 0.80 at 500 nm in 1 cm light path. Standard is stable until expiration date on label when stored at 2-30°C. Reagent and standard should be at room temperature before use.

NOTE: To prevent contamination of Glucose reagent, pour into a separate vessel a volume slightly in excess of that required. DO NOT RETURN UNUSED PORTION.

Materials Required But Not Provided

Spectrophotometer capable of reading at 500 nm (492-530 nm)
Accurate pipetting devices
Heating block or water bath, 37°C
Cuvets ; Vortex ; Mixer ; Interval Timer

Specimen Collection and Preparation

Serum: Remove from clot within 30 minutes of collection in order to prevent glycolysis.
Plasma: An anticoagulant containing fluoride is recommended, but any of the common anticoagulants may be used if plasma is separated from cells promptly after centrifugation.
CSF: No special preparation is required.
Sample Stability: Glucose in serum/plasma processed in the manner described is stable for 40 hours at 2-8°C. CSF samples should be analyzed immediately because of possible bacterial contamination.
Interfering Substances: False low glucose values can result from excessive levels of ascorbic acid.[7]

Automated Analyzers

Parameters:

Wavelength	500 nm
Reaction Type	Endpoint
Reaction Direction	Increasing
Reaction Temperature	37°C
Sample/Reagent Ratio	1:100
Equilibration Time	3 Seconds
Read Time	4 Seconds
Lag Time	300 Seconds
Blank Absorbance Limit	0.800
High Absorbance	2.000A
Standard	100 mg/dL
Low Normal	70 mg/dL
High Normal .	105 mg/dL
Linearity	400 mg/dL

Above parameters should be employed in programming automated analyzers for Glucose. Consult your instrument manual for programming instructions. Specific programming applications for most automated analyzers are available from Interchim Customer Service Department.

Manual Procedure

Procedure #1: (Linear to 400 mg/dL)*
1. Pipet into cuvets the following volumes (mL) and mix well:

	Reagent Blank (RB)	Standard (S)	Sample (U)
Reagent	1.0	1.0	1.0
Standard	–	0.010	–
Sample	–	–	0.010

*If linearity is desired to 500 mg/dL, increase reagent volume to 1.5 mL and proceed using 0.010 of sample.
2. Incubate all cuvets at 37°C for 5 minutes.
3. Read S and U vs RB at 500 nm.

Procedure #2: (Linear to 650 mg/dL)
1. Pipet into cuvets the following volumes (mL) and mix well:

	Reagent Blank (RB)	Standard (S)	Sample (U)
Reagent	1.0	1.0	1.0
Standard	–	0.005	–
Sample	–	–	0.005

NOTE: Volumes may be increased if the instrument requires a volume greater than 1.0 mL.
2. Incubate all cuvets at 37°C for 5 minutes.
3. Read S and U vs RB at 500 nm.

Quality Control: Two levels of control material with known glucose levels determined by this method or a hexokinase procedure should be analyzed each day of testing. Interchim recommends, Ser-T-Fy® I, Normal Control # FT7670 and Ser-T-Fy® II, Abnormal Control # FT7680 be assayed with each patient run.

Results

Values are derived by the following equations:
Glucose (mg/dL) = $\frac{Au}{As} \times 100$,
where Au and As are the absorbance values of UNKNOWN and STANDARD, respectively and 100 the concentration of the STANDARD (mg/dL).
Example: Following readings were obtained using 1 cm cuvets: Au = 0.130, As = 0.178
Glucose (mg/dL) = $\frac{0.130}{0.178} \times 100 = 73$
Glucose (mmol/L) = Glucose (mg/dL) x 0.0556
NOTE: Samples having glucose values greater than 500 mg/dL are diluted 1:2 (1 + 1) with distilled water, the assay repeated and results multiplied by the dilution factor 2.

Expected Values[8]

Normal Range: Serum/Plasma: 70-105mg/dL
CSF: 40-75 mg/dL (2.2-3.9 mmol/L)
It is recommended that each laboratory establish its own range of expected values, since differences exist between instruments, laboratories, and localpopulations.

Performance Characteristics[9]

Precision: Using a serum containing glucose in the normal range and one with elevated values, a series of 5 assays were performed on each of 5 days. Coefficients of variation (CV) were within run of 1.6 and 1.2%, and between runs 3.0 and 2.0%, respectively.
Correlation: Determination of glucose by the procedure described (y) and by a hexokinase/UV method (x) on 40 sera (range: 56-582 mg/dL) showed a correlation coefficient (r) of 0.995 and a regression equation of y = 0.98x - 1.99.
Linearity: When performed as directed, the method is linear as listed for each procedure.

References

1. Folin O., Wu H.: J Biol Chem 41:367, 1920
2. Keilin D., Hartree E.F.: Biochem J 42:230, 1948
3. Keston AS: Abstr 129th Meeting, Am Chem Soc, 1956, p 31c.
4. Teller J.D.: Abstr 130th Meeting, Am Chem Soc, 1956, p 69c.
5. Trinder, P., "Determination of Blood Glucose Using 4-Aminophenazone." J. Clin. Path., 22:246 (1959).
6. Howanitz P.J., Howanitz J.H.: IN Clinical Diagnosis and Management by Laboratory Methods, 17th ed., J.B. Henry, Ed., W.B. Saunders, Philadelphia, 1984, p 168.
7. Cooper G.R., McDaniel V: Manual of Methods for the Determination of Glucose, CDC, USPHS, Atlanta.
8. Caraway W.T.: IN Fundamentals of Clinical Chemistry, 2nd ed., N.W. Tietz, Ed. Saunders, Philadelphia, 1976, p 242.
9. Interchim Laboratory data

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