Manual Procedure

Automated procedure on request

MEDICHEM MIDDLE EAST
Clinical Chemistry Reagents
Liquid Stable Reagents

Cat. No.12410 R 1 x 50 ml
For 50 tests

Cat. No.12411 R 3 x 50 ml
For 150 tests

Glucose HK

Enzymatic UV method

Liquid Reagent

Test Principle

Glucose present in the sample is determined according to the following reactions:

$$\text{Glucose} + \text{ATP} \xrightarrow{HK} G_6P + \text{ADP}$$

$$G_6P + \text{NADP} \xrightarrow{G6PDH} \text{6-Phosphogluconate} + \text{NADPH} + H^+$$

Glucose is phosphorylated with adenosine triphosphate (ATP) in the reaction catalyzed by hexokinase (HK). The product, glucose-6-phosphate (G_6P), is then oxidized with the concomitant reduction of nicotinamide adenine dinucleotide (NADP) to NADH in a reaction catalyzed by glucose-6-phosphate-dehydrogenase (G6PDH). The formation of NADPH causes an increase in absorbance at 340 nm which is directly proportional to the amount of glucose present in the sample.

Concentrations in the test

Reagent R (pH = 7.60)		
PIPES	100	mmol/L
Mg^{+2}	23.3	mmol/L
NADP	3.8	mmol/L
ATP	9.2	mmol/L
Hexokinase	≥ 4	KU/L
G6PDH	≥ 4	KU/L
Preservative, Detergent.		
Standard : The Concentration as indicated on vial.		

Stability of reagent

Reagent R : *liquid, ready to use.*
The reagent is stable up to expiry date given on the label when stored at +2 ➔ +8°C.

Note: Don't use the reagent if it has an absorbance greater than 0.500 against dist. water at 340 nm, or contains obvious microbial growth.

Specimen collection and handling

1. Fresh, non-hemolyzed serum.
2. Non-hemolyzed plasma collected on oxalate, citrate, EDTA, fluoride or heparin.
3. Serum and plasma must be separated from the red cells promptly to prevent glycolysis. Glucose will decrease approximately 7 % per hour when left in contact with red cells.
4. Glucose in serum is stable for 24 hours at 15 - 25 °C and for 7 days at 2 - 8 °C, after addition of a glycolysis inhibitor (NaF, KF).
5. Cerebrospinal fluid (CSF) could be used as obtained.
6. Use fluoride plasma in case of a delay in analysis.

Calibrator / Standard

MediCal U Cat. No. 15011
Glucose STD. Cat. No. 16111

Quality control

Meditrol N Cat. No. 15171
Meditrol P Cat. No. 15181

Procedure

Wavelength	Hg 340 (334 - 365 nm)
Spectrophotometer	340 nm
Cuvette	1 cm light path
Temperature	37°C / 20 - 25 °C
Measurement	against blank
Reaction	end point

Assay

	Blank	Calibrator/ Standard	Sample
Distilled water	10 µl	--	--
Calibrator / Standard	--	10 µl	--
Sample	--	--	10 µl
Reagent R	1000 µl	1000 µl	1000 µl
Mix, incubate for 5 min. at 37 °C or 10 min. at 20 - 25 °C, Read the absorbance (A). The absorbance is stable for 1 hour.			

Calculation

$$\text{Conc.}_{\text{Glucose}}\ (\text{mg/dl}) = \frac{A_{\text{Sample}}}{A_{\text{Cal./STD.}}} \times \text{Conc.}_{\text{Cal./STD.}}\ (\text{mg/dl})$$

$$\text{mmol/L} \underset{0.0555}{\overset{18}{\rightleftharpoons}} \text{mg/dl}$$

Linearity

Up to 500 mg/dl (27.75 mmol/L).
If the result exceeds 500 mg/dl, repeat the test using diluted sample (1+2) with sodium chloride solution (0.9 %) and multiply the result by 3.

Interferences

1. Bilirubin to the level of 20 mg/dl has been found to exhibit negligible interference (~ 5 %) in this assay.
2. Hemoglobin to the level of 400 mg/dl has been found to exhibit negligible interference (~ 5 %) in this assay.
3. A number of drugs and substances affect glucose results. see Young,*et al.*[4]

Precaution

The reagent contains sodium azide as a preservative. Avoid contact with skin and mucous membranes. Sodium azide may form explosive compounds in metal drain lines. When disposing of reagent through plumbing fixtures, flush with copious amounts of water.

Reference range

Serum	Adults	75 - 115	mg/dl
Plasma (venous)	Adults	55 - 115	mg/dl
CSF	Adults	50 - 70	mg/dl

References

1. Hoffmeister, H. U.,Junge, B.,Z. Clin. Chem. Clin. Biochem, 8(1970) 613.
2. R. J. and M. Henry, Clin. Chem, 2. Aufl., Harper Row New York 1974, S. 1301 – 1302.
3. L. Thomas, labor and diagnose, med. Verlagsgesellschaft, Marburg / Lahn, 1978, S. 167 – 180.
4. Young, DS., Effects of Drugs on Clinical Laboratory Tests, fifth edition 2000, AACC Press, Washington, D.C.