Glucose HK

For glucose determination in serum, plasma, urine or cerebrospinal fluid

SUMMARY

Glucose determination in blood is generally used in the diagnosis of carbohydrates metabolism disorders including diabetes mellitus among them. Early diagnosis and monitoring of diabetic patients are aimed to prevention of both ketoacidosis as well as complications of the symptoms coming from hyperglycemia, by using a proper therapy. Since there are many causative factors of hyperglycemia (i.e. hyperthyroidism or corticoadrenal hyperfunction) or hypoglycemia (i.e. excessive insulin therapy, neonatal hypoglycemia, pancreatic islet cells cancer or hepatic disorders), should be considered individually, the physiological conditions and/or the patient' disease.

PRINCIPLE

The reaction scheme is the following:

$$\text{Glucose} + \text{ATP} \xrightarrow{\text{HK}} \text{Glucose-6-Phosphate} + \text{ADP}$$

$$\text{Glucose-6-P} + \text{NAD}^+ \xrightarrow{\text{G-6-PDH}} \text{Gluconate-6-P} + \text{NADH} + \text{H}^+$$

PROVIDED REAGENTS

A. Reagent A: 100 mM Tris buffer solution, 4 mM magnesium salt, ≥ 1.7 mM ATP, ≥ 1.5 mM NADP, 0.9 g/l sodium azide, pH 7.8.
B. Reagent B: 30 mM Goods buffer solution, 4 mM magnesium salt, ≥ 5 U/ml hexokinase, ≥ 10 U/ml glucose-6-phosphate dehydrogenase, 0.9 g/l sodium azide, pH 7.0.
S. Standard*: 100 mg/dl (1 g/l) glucose solution.

NON-PROVIDED REAGENTS

- Distilled or demineralized water
- Saline solution (9 g/l ClNa)
- Wiener lab's **Calibrador A plus**

INSTRUCTIONS FOR USE

Provided Reagents: ready to use.

WARNINGS

Reagents are for "in vitro" diagnostic use.
Do not exchange reagents from different lots.
All patient samples should be handled as capable of transmitting infection.
Use the reagents according to the working procedures for clinical laboratories.
Reagents and samples should be discarded according to the local regulations in force.

STABILITY AND STORAGE INSTRUCTIONS

Provided Reagents: stable at 2-10ºC until the expiration date stated on the box. Do not store at elevated temperatures for extended periods of time.
Once opened, do not keep outside refrigerator for extended periods of time. Avoid contamination.

INSTABILITY OR DETERIORATION OF REAGENTS

Evident signs of spilling, turbidity, coloration, microbial growth or quality control results outside acceptable ranges indicate reagent deterioration. Discard in such case.

SAMPLE

Serum, plasma, urine or cerebrospinal fluid (CSF)
a) Collection:
- Serum or plasma: obtain serum in the usual way, by checking the complete clot formation. If plasma is used, collect with ordinary anticoagulants, centrifuging sample before testing.
- Urine: if the urine specimen is a random sample, preferably use fresh urine. If the assay cannot be performed immediately, store sample at 2-10ºC. Urine testing may be performed within 24 hours. In such case, collect the urine in a dark container with 5 ml of glacial acetic acid and store in ice.
- CSF: if CSF is used, perform the assay immediately after sample collection.

b) Additives: if plasma is used as sample, the use of Wiener lab's **Anticoagulante G** (EDTA, fluoride) or **Anticoagulante W** (EDTA) or < 20 IU/ml sodium heparin is recommended.
c) Known interfering substances: no significant interferences have been observed with unconjugated bilirubin up to 45 mg/dl or conjugated bilirubin up to 40 mg/dl, lipids up to 2500 mg/dl triglyceride concentrations or hemolysis up to 1500 mg/dl hemoglobin concentrations.
In urine samples, no significant interferences have been observed with 1000 mg/dl ascorbic acid concentrations.
See Young, D.S. in References for effect of drugs on the present method.
For diagnostic purposes, results should always be jointly evaluated with the patient's clinical background, clinical examination and other findings.
d) Stability and storage instructions: the enzymatic destruction of blood glucose (glycolysis) by red blood cells and leukocytes is proportional to the temperature at which blood is stored, reaching its maximum at 37ºC. This process is not even inhibited by freezing. Therefore, blood should be centrifuged within 60 minutes after collection until a clear

** Non-provided with all kit sizes*

supernatant is obtained. Transfer to another tube for storage. In this manner, glucose is stable for up to 4 hours at room temperature or for up to 24 hours refrigerated. If unable to process the sample as indicated above, add a preservative to blood when collecting. In stored samples, observe the presence of particles. If they are present, mix and centrifuge sample for removing before testing.

REQUIRED MATERIAL (non-provided)
- Spectrophotometer
- Micropipettes and pipettes for measuring stated volumes
- Square spectrophotometer cuvettes
- Water bath at 37°C
- Watch or timer

ASSAY CONDITIONS
- Wavelength: 340 nm (primary) in spectrophotometer (380 nm as secondary wavelength in automatic analyzer)
- Reaction temperature: 37°C
- Reaction time: 9 minutes
- Sample volume: 20 ul
- Reagent A volume: 1.5 ml
- Reagent B volume: 0.3 ml
- Final reaction volume: 1.82 ml

Sample and Reagent volumes may change proportionally (i.e. 600 ul Reagent A, 8 ul Sample and 120 ul Reagent B)

PROCEDURE

In two test tubes labeled S (Standard or Calibrator) and U (Unknown) placed in water bath at 37°C add:

	S	U
Reagent A	1.5 ml	1.5 ml
Standard or Calibrator	20 ul	-
Sample	-	20 ul

Measure in spectrophotometer at 340 nm, previously taking the instrument to 0 with distilled or demineralized water. Label this measure as A_1 (Sample Blank). In the same water bath, without removing the test tube, add Reagent B:

Reagent B	0.3 ml	0.3 ml

Mix and incubate in water bath at 37°C for seven (7) minutes. Measure in spectrophotometer at 340 nm and label this measure as A_2.

STABILITY OF FINAL REACTION

Final absorbance should be measured within 20 minutes.

CALCULATIONS

Correct the obtained A_2 measurements, subtracting the corresponding sample blanks (A_1):

$S = A_{2S} - A_{1S}$

$U = A_{2U} - A_{1U}$

Glucose (mg/dl) = U x f

$$f = \frac{100 \text{ mg/dl or } C}{S}$$

where

C = glucose concentration in Calibrador A plus

To express glucose result in 24-hour urine in g/24 hrs:

$$\text{Glucose (g/24 hrs)} = \frac{V \times c}{100}$$

where:

V = 24-hour urine volume (in liters)

c = glucose concentration (mg/dl)

QUALITY CONTROL METHOD

Each time the assay is performed, test two levels of quality control material (**Standatrol S-E 2 niveles**) with known glucose concentration.

REFERENCE VALUES

In Tietz, N.W. bibliography, the following Reference Value range is mentioned:

Serum or plasma

Adults: 74-106 mg/dl (4.11-5.89 mmol/l)
Children: 60-100 mg/dl (3.33-5.55 mmol/l)
Newborns: 1 day old: 40-60 mg/dl (2.22-3.33 mmol/l)
> 1 day old: 50-80 mg/dl (2.78-4.44 mmol/l)

Fresh random urine

1-15 mg/dl (0.06-0.83 mmol/l)

24-hour urine

< 0.5 g/24 hrs (< 2.78 mmol/24 hrs)

CSF

Children: 60-80 mg/dl (3.33-4.44 mmol/l)
Adults: 40-70 mg/dl (2.22-3.89 mmol/l)

Each laboratory should establish its own reference values considering age, sex, dietary habits, medication and other population factors.

SI SYSTEM UNITS CONVERSION

Glucose (mg/dl) x 0.0555 = Glucose (mmol/l or mM)
Glucose (g/24 hours) x 55.5 = Glucose (mmol/24 hrs)

PROCEDURE LIMITATIONS

See Known interfering substances and Stability and storage instructions under SAMPLE.

PERFORMANCE

The assays were performed in Wiener lab CT600i analyzer.
a) Precision: 3 activity levels were tested by duplicate in two daily runs during 20 days. Intra-assay and total precision were calculated.

Intra-assay precision

Level	C.V.
86.8 mg/dl	0.56 %
195.1 mg/dl	0.65 %
290.7 mg/dl	0.64 %

Total precision

Level	C.V.
86.8 mg/dl	1.87 %
195.1 mg/dl	1.76 %
290.7 mg/dl	1.64 **%**

b) Linearity: reaction in serum or plasma is linear from 5 to 100 mg/dl glucose (0.28 to 55.50 mmol/l). In urine is linear from 4 to 2000 mg/dl glucose (0.22 to 111.0 mmol/l). For higher values, dilute sample using saline solution. Repeat the assay following the reaction conditions and multiply final result by the dilution factor.
e) Sensitivity: detection limit is 0.5 mg/dl and analytical sensitivity is 4.4 mg/dl.

PARAMETERS FOR ANALYZERS
For programming instructions check the user manual of the analyzer to be used.
Wiener lab's **Calibrador A plus** should be used for calibration, following the analyzer requirements.

WIENER LAB PROVIDES
- 8 x 50 ml Reagent A + 4 x 20 ml Reagent B (Cat. Nº 1009618)
- 8 x 50 ml Reagent A + 4 x 20 ml Reagent B (Cat. Nº 1009926)
- 8 x 55 ml Reagent A + 8 x 11 ml Reagent B (Cat. Nº 1009314)

REFERENCES

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- Zensuke Ogawa, Motohito Kanashima and Hiroshi Nishioka - Clin. Chem. Lab. Med. 39/5:396, 2001.
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- Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline. CLSI EP17-A Vol. 24 Nº 34, 2004.

SYMBOLS
The following symbols are used in the packaging for Wiener lab. diagnostic reagents kits.

CE	This product fulfills the requirements of the European Directive 98/79 EC for "in vitro" diagnostic medical devices
EC REP	Authorized representative in the European Community
IVD	"In vitro" diagnostic medical device
Σ	Contains sufficient for <n> tests
	Use by
	Temperature limitation (store at)
	Do not freeze
	Biological risks
⟶	Volume after reconstitution
Cont.	Contents
LOT	Batch code
	Manufactured by:
	Harmful
	Corrosive / Caustic
	Irritant
	Consult instructions for use
Calibr.	Calibrator
CONTROL	Control
CONTROL +	Positive Control
CONTROL −	Negative Control
REF	Catalog number

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