Glicemia
enzimática AA

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

SUMMARY
Diabetes mellitus is the pathology most commonly related to carbohydrates metabolism. Early diagnosis and the periodic check of diabetic patients are aimed to prevent both ketoacidosis as well as complications of the symptoms coming from hyperglycemia, by means of a proper therapy.
Due to the existence of many causative factors of hypo- or hyperglycemia, physiological conditions and specific pathological features should be individually considered for each patient.

PRINCIPLE
The reaction system is as follows:

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{GOD}} \text{Gluconic Acid} + H_2O_2$$

$$2\,H_2O_2 + \text{4-AP} + \text{4-Hydroxybenzoate} \xrightarrow{\text{POD}} \text{quinoneimine}$$

PROVIDED REAGENTS
S. Standard: 100 mg/dl (1 g/l) glucose solution.
A. Reagent A: vials containing glucose oxidase (GOD), peroxidase (POD) and 4-aminophenazone (4-AP).
B. Reagent B: phosphates buffer pH 7.0 containing 4-hydroxy-benzoate.

Final concentrations

GOD (microbial)	≥ 10 kU/l
POD (horse-radish)	≥ 1 kU/l
4-AP	0.5 mmol/l
Phosphates	100 mmol/l, pH 7.0
4-Hydroxybenzoate	12 mmol/l

NON-PROVIDED REAGENTS
Wiener lab.'s **Calibrador A plus**.

INSTRUCTIONS FOR USE
Standard: ready to use.
Working Reagent: reconstitute the content of a vial of Reagent A with a part of Reagent B. Transfer to the Reagent B bottle, rinsing the vial several times with it. Mix until complete dissolution. Homogenize and date.

WARNINGS
Reagents are for “in vitro” diagnostic use.
Use the reagents according to the working procedures for clinical laboratories.
The reagents and samples should be discarded according to the local regulations in force.

STABILITY AND STORAGE INSTRUCTIONS
Provided Reagents: stable in refrigerator (2-10ºC) until the expiration date shown on the box. Avoid exposure to high temperatures for long periods.
Working Reagent: in refrigerator (2-10ºC) is stable for 60 days from preparation date.

INSTABILITY OR DETERIORATION OF REAGENTS
During its use, the Working Reagent may develop a light pink coloration which does not affect its performance as long as a Blank is processed for each determination lot and a Standard at least once a week. Discard when the Blank readings are higher than 0.160 O.D.

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: when using plasma, it is recommended to use heparin or Wiener lab.s' **Anticoagulante G** (EDTA/fluoride).
c) Known interfering substances: no interferences are observed from bilirubin up to 10 mg/dl, triglycerides up to 500 mg/dl and hemoglobin up to 350 mg/dl. The ascorbic acid interferes with any concentration of urine determination. See Young, D.S. in References for effect of drugs on the present method.
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 inhibited by freezing. Therefore, blood should be centrifuged within 2 hours after collection until a clear supernatant is obtained. Transfer to another tube for storage. Glucose is stable for 4 hours at room temperature or 24 hours refrigerated. When it is not possible to process the sample as indicated above, add a preservative to blood when collecting.
The CSF may be contaminated with bacteria and other cells.

Thus, the test should be immediately performed. Otherwise, centrifuge the CSF and store for 3 days at 2-10°C or for 5 hours at 20-25°C.

REQUIRED MATERIAL (non-provided)
- Spectrophotometer or photocolorimeter.
- Micropipettes and pipettes for measuring the stated volumes
- tubes or spectrophotometric cuvettes.
- Water bath at 37°C.
- Watch or timer.

ASSAY CONDITIONS
- Wavelength: 505 nm in spectrophotometer or in photocolorimeter with green filter (490-530 nm).
- Reaction temperature: 37°C
- Reaction time: 5 minutes
- Sample volume: 10 ul
- Working Reagent volume: 1 ml
- Final reaction volume: 1.01 ml

Sample and Reagent volumes may proportionally vary (e.g. 20 ul Sample + 2 ml Working Reagent).

PROCEDURE

In three test tubes labeled B (Blank), S (Standard) and U (Unknown) place:

	B	S	U
Standard	-	10 ul	-
Sample	-	-	10 ul
Working Reagent	1 ml	1 ml	1 ml

Incubate tubes for 5 minutes in water bath at 37°C or for 25 minutes at 15-25°C. Read in spectrophotometer at 505 nm or in photocolorimeter with green filter at 490-530 nm, setting instrument to zero O.D. with Blank.

STABILITY OF FINAL REACTION

Final reaction color is stable for 30 minutes, therefore absorbance should be read within that period.

CALCULATIONS

$$\text{Glucose (mg/dl)} = U \times f \qquad f = \frac{100 \text{ mg/dl}}{S}$$

QUALITY CONTROL METHOD

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

REFERENCE VALUES

In a study performed with Wiener lab.'s **Glicemia enzimática AA** among 120 fasting individuals from Rosario (Argentina), from both sexes (between 20 and 45 years old), without presenting symptoms of diabetes or other diseases, 95% of results, cover this range:

Serum or plasma: 70 - 110 mg/dl

In the literature (Tietz, N.W.) 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)

It is recommended that each laboratory establishes its own reference values for their own patients, considering age, sex, dietary habits and other factors.

SI SYSTEM UNITS CONVERSION

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

PROCEDURE LIMITATIONS

See Known Interfering Substances under SAMPLE.

PERFORMANCE

The assays were performed in an Express plus analyzer(*).
a) Reproducibility: testing 20 replicates from the same sample in 5 different days, the following results were obtained:

Intra-assay precision

Level	S.D.	C.V.
90.7 mg/dl	±1.26 mg/dl	1.39 %
278 mg/dl	± 3.08 mg/dl	1.11 %

Inter-assay precision

Level	S.D.	C.V.
90.1 mg/dl	± 1.73 mg/dl	1.92 %
299 mg/dl	± 4.86 mg/dl	1.62 %

b) Recovery: adding known amounts of glucose to different sera, a recovery between 99 and 101% was obtained.
c) Linearity: reaction is linear up to 500 mg/dl. For higher values, dilute the sample with saline solution, repeat the test and multiply final result by the dilution factor.
d) Correlation: glucose values of 154 serum specimens covering a range from 23 mg/dl to 503 mg/dl were determined using the Wiener lab's **Glicemia enzimatica AA** kit and a commercial kit based on same principle. The correlation coefficient was: r = 0.9997, slope b = 1.0257 and intercept a = 1.9485.
e) Sensitivity studies: minimum detection limit is 0.54 mg/dl and analytical sensitivity is 4.2 mg/dl.

PARAMETERS FOR AUTOANALYZERS

For programming instructions check the user manual of the

autoanalyzer in use.
For calibration can be use Wiener lab's **Calibrador A plus**, following the autoanalyzer requirements.

WIENER LAB PROVIDES
- 1 x 250 ml (Cat. Nº 1400106).
- 4 x 250 ml (Cat. Nº 1400107).

REFERENCES

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SYMBOLS
The following symbols are used in 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

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:

Xn — Harmful

Corrosive / Caustic

Xi — Irritant

Consult instructions for use

Calibr. — Calibrator

CONTROL — Control

CONTROL + — Positive Control

CONTROL − — Negative Control

REF — Catalog number

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