Glicemia

enzimática

Enzymatic method for glucose determination in serum or plasma

SUMMARY

Diabetes mellitus is the pathology most commonly related to carbohydrates metabolism. Early diagnosis and the periodic monitoring 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:

$$\text{glucose} + O_2 + H_2O \xrightarrow{\text{GOD}} \text{gluconic acid} + H_2O_2$$

$$2\,H_2O_2 + \text{4-AP} + \text{phenol} \xrightarrow{\text{POD}} \text{colored quinone} + 4\,H_2O$$

PROVIDED REAGENTS

A. Reagent A: 25 mmol/l 4-aminophenazone solution in 0.92 mol/l Tris Buffer.
B. Reagent B: 55 mmol/l phenol solution.
C. Reagent C: glucose oxidase solution (1000 U/ml) and peroxidase (120 U/ml).
S. Standard: 1 g/l glucose solution.

Final concentrations

GOD	≥ 3000 U/l
POD	≥ 400 U/l
4-AP	1.25 mM
Phenol	2.75 mM
pH	7.4 ± 0.1

NON-PROVIDED REAGENTS

Distilled water. See PROCEDURE LIMITATIONS

INSTRUCTIONS FOR USE

Standard: ready to use.
Reagent A: ready to use.
Reagent B: ready to use. See WARNINGS.
Reagent C: mix by inversion before use, avoiding foam formation.
Working Reagent: according to the volume to prepare, in a test tube place 500 parts of distilled water, 50 parts of Reagent A, 50 parts of Reagent B and take to 1000 parts with distilled water. Add 3 parts of Reagent C previously homogenized. Mix by inversion without stirring. Label and date. Different quantities can be prepared following the above proportions. It is also important to respect the order of addition of the reagents and ensure their perfect homogenization, so that Reagent B does not deteriorate the Working Reagent.

WARNINGS

Reagents are for “in vitro” diagnostic use.
Reagent B (phenol) is harmful and corrosive. H301 + H311 + H331: Toxic if swallowed, in contact with skin or if inhaled. H314: Causes severe skin burns and eye damage. P262: Do not get in eyes, on skin, or on clothing. P305 + P351 + P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P302 + P352: IF ON SKIN: Wash with plenty of soap and water. P280: Wear protective gloves/protective clothing/ eye protection/face protection. Use reagents maintaining the usual work precautions in the clinical laboratory. All reagents and samples must be discarded according to local regulations in force.

STABILITY AND STORAGE INSTRUCTIONS

Provided Reagents: stable in refrigerator (2-10°C) until the expiration date shown on the box. Avoid exposure to high temperatures for extended periods of time.
Working Reagent: in refrigerator (2-10°C) and in caramel-colored bottle it is stable for one month from preparation date.

INSTABILITY OR DETERIORATION OF REAGENTS

During use, the Working Reagent may develop a light pink coloration which does not affect its performance as long as, periodically, a Blank is processed for each lot of determination and a Standard is periodically used. Discard when the Blank readings are higher than 0.160 O.D. or the Standard readings are abnormally low.

SAMPLE

Serum or plasma
a) Collection: obtain serum or plasma as usual. When it is impossible to withdraw venous blood or in cases of extreme urgency, the test can be performed in capillary blood.
b) Additives: when using plasma, the use of Wiener lab’s **Anticoagulante G** is recommended for collection (it contains fluoride as preservative).
c) Known interfering substances: sera or plasmas with visible or intense hemolysis should be deproteinized. No interferences are observed with: bilirubin up to 200 mg/l, ascorbic acid up to 75 mg/l, uric acid up to 200 mg/l, slight hemolysis. Refer to Young, D.S. in References for the effect of drugs on the present method.
d) Stability and storage instructions: red blood cells and leukocytes are responsible for the enzymatic destruction of

blood glucose, reaching its maximum at 37°C. Therefore, blood should be centrifuged within 2 hours after collection until a clear supernatant is obtained and transfer to another tube for storage. In these conditions, glucose is stable for 4 hours at room temperature or for 24 hours refrigerated (2-10°C). When it is impossible to process the sample as indicated above, add a preservative to blood at collection to inhibit glycolysis.

REQUIRED MATERIAL (non-provided)
- Spectrophotometer or photocolorimeter.
- Suitable volumetric material.
- Caramel-colored glass bottle.
- Tubes or square 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: 10 minutes
- Sample volume: 20 ul
- Working Reagent volume: 2 ml
- Final reaction volume: 2.02 ml

Sample and Reagent volumes may proportionally vary (e.g. 50 ul Sample + 5 ml Working Reagent).

PROCEDURE

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

	B	S	U
Standard	-	20 ul	-
Sample	-	-	20 ul
Working Reagent	2 ml	2 ml	2 ml

Incubate for 10 minutes in water bath at 37°C. Then read in spectrophotometer at 505 nm or in photocolorimeter with green filter (490-530 nm) setting the instrument to zero O.D. with the Blank.

STABILITY OF FINAL REACTION

Final reaction color is stable for 1 hour therefore absorbance should be read within that period.

CALCULATIONS

$$\text{Glucose g/l} = U \times f \quad \text{where } f = \frac{1.00 \text{ g/l}}{S}$$

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 **Glicemia enzimática** among 120 fasting individuals from Rosario (Argentina), from both sexes, aged between 20 and 45 years old, without presenting symptoms of diabetes or other diseases, 95% of the results covered this range:

Serum or plasma: 0.70 - 1.10 g/l

In the bibliography (Tietz, N.W.) the following reference range is mentioned:

Serum or plasma: 0.74 - 1.06 g/l

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

UNITS CONVERSION

Glucose (g/l) = Glucose (g/l) x 0.01
Glucose (mg/dl) x 0.0555 = Glucose (mmol/l)

PROCEDURE LIMITATIONS

See Known Interfering Substances under SAMPLE. Reducing agents decrease the color response, while oxidants color the Reagent increasing the Blanks. These agents are often found in the distilled water used to prepare the Working Reagent, so it is recommended to monitor water quality. Detergents, heavy metals and cyanides are enzymatic inhibitors.

PERFORMANCE

a) Reproducibility: testing replicates from the same sample in 10 different days, the following results were obtained:

Level	S.D.	C.V.
1.00 g/l	± 0.022 g/l	2.37 %
2.00 g/l	± 0.030 g/l	1.50 %

b) Recovery: by adding known amounts of glucose to various sera, a recovery between 99% and 101% was obtained.
c) Linearity: reaction is linear up to 4.5 g/l. For higher values, dilute ½ the final colored solution with the Working Reagent and repeat the reading multiplying the final result by two.
d) Accuracy: using the hexokinase method as reference it was noted that the statistical correlation between methods was excellent (r = 0.99).
e) Sensitivity: the minimum detection limit is 0.0054 g/l and the analytical sensitivity is 0.042 g/l.

WIENER LAB PROVIDES

- 1000 ml (Cat. No. 1400101).

REFERENCES

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Symbols

The following symbols are used in the packaging for Wiener lab. diagnostic reagent 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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