LIQUID LINE

Fructosamina

AA

Colorimetric method (NBT) for fructosamine determination in serum or plasma

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.

Glycated proteins (fructosamine) are formed by covalent link of glucose with lysine residues from blood proteins (albumin mainly), forming Schiff bases, followed by an irreversible transformation to ketoamines (fructosamine).

This reaction depends on the blood glucose concentration and the protein interaction time. Fructosamines stay in blood acting as "glycemic memory" until their metabolization, analog to that of with the other serum proteins. As a result, fructosamine concentration retrospectively reflects the average blood glucose concentration, two or three weeks before the test is performed.

PRINCIPLE

The method is based on the ability of the ketoamine group of glycated proteins to reduce tetrazolium salt (NBT) to formazan in alkaline medium. The formazan is photocolorimetrically measured at 530 nm. Its rate formation is proportional to the fructosamine concentration in sample.

PROVIDED REAGENTS

A. Reagent A: 0.25 mmol/l nitroblue tertrazolium (NBT) solution in 0.2 mol/l carbonate buffer.

S. Standard*: lyophilized serum containing a fructosamine concentration between 200-700 umol/l of glycated albumin (1.7-6.1 mmol/l DMF, deoximorpholinofructose). The concentration, that is lot specific, is given on the label.

NON-PROVIDED REAGENTS

Bidistilled or deionized water.

INSTRUCTIONS FOR USE

Reagent A: ready to use.

Standard: reconstitute with 1 ml distilled water measured with precision micropipette or pipette with double calibration mark. Cap and mix gently by inversion. Do not shake. Let stand for 60 minutes at room temperature, sporadically mixing by inversion. Date. Homogenize by inversion before use.

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. Store the Reagent A avoiding light exposure.

Reconstituted Standard: stable for 15 days in refrigerator (2-10ºC) or for 45 days frozen (-20ºC) and aliquoted.

SAMPLE

Serum or plasma.

a) Collection: obtain serum in the usual way or plasma with heparin or EDTA. See REFERENCE VALUES.

b) Known Interfering Substances: samples with visible or intense hemolysis should not be used. No interferences are observed from triglycerides up to 10 g/dl, bilirubin up to 20 mg/l, uric acid up to 150 mg/l and mild hemolysis. See Young, D.S. in References for effect of drugs on the present method.

c) Stability and Storage instructions: samples should be preferably fresh. In case the test cannot be performed on the same day, the samples may be stored for 7 days in refrigerator (2-10ºC) or 2 months frozen (-20ºC).

REQUIRED MATERIAL (non-provided)

- Spectrophotometer or photocolorimeter.
- Micropipettes and pipettes for measuring the stated volumes
- Tubes or spectrophotometric square cuvettes.
- Water bath at 37ºC.
- Stopwatch.

ASSAY CONDITIONS

- Wavelength: 530 nm in spectrophotometer or in photocolorimeter with green filter (490 - 530 nm)
- Reaction temperature: 37ºC
- Reaction time: 15 minutes
- Sample volume: 50 ul
- Final reaction volume: 1.05 ml

Sample and Reagent A volumes can be proportionally changed (e.g. 100 ul Sample + 2 ml Reagent A)

PROCEDURE

In two test tubes labeled S (Standard) and U (Unknown) place:

** Non-provided with all kit sizes*

	S	U
Standard	50 ul	-
Sample	-	50 ul
Reagent A	1 ml	1 ml

Mix thoroughly and place in water bath at 37°C. Immediately start stopwatch. Read Sample and Standard absorbances after 10 minutes (S_1 or U_1) and after 15 minutes (S_2 or U_2) in spectrophotometer at 530 nm or in photocolorimeter with green filter (490-530 nm), setting the instrument to zero with distilled water.

CALCULATIONS

The absorbance difference between both readings is proportional to the fructosamine concentration. Thus, the calculation is the following:

Fructosamine (umol/l or mmol/l) = $(U_2 - U_1) \times f$

$$f = \frac{C^*}{S_2 - S_1}$$

*Standard concentration in umol/l (glycated albumin) or mmol/l (DMF)

QUALITY CONTROL METHOD

Wiener lab.'s **Fructosamina Control 2 niveles**.

REFERENCE VALUES

205-285 umol/l (glycated albumin)
1.9-2.9 mmol/l (DMF)

It is recommended that each laboratory establishes its own intervals and reference values according to age, sex, dietary habits and other factors.
Decreased values may be observed in patients with considerable albumin loss or protein catabolism diseases.
Plasmatic fructosamine levels are slightly lower than serum fructosamine levels.

PROCEDURE LIMITATIONS

See Known Interfering Substances under SAMPLE.
Reducing agents decrease color response, while oxidizing agents color the Reagent increasing the Blanks.
It is recommended to perform one weekly recalibration or each time values outside the control's acceptable range are obtained (Fructosamina Control 2 niveles).

PERFORMANCE

a) Reproducibility: when 20 replicates of the same samples were assayed on the same day, the following results were obtained:

Level	C.V.
265 umol/l (2.3 mmol/l)	1.3 %
731 umol/l (6.3 mmol/l)	0.7 %

b) Linearity: reaction is linear up to 800 umol/l (7 mmo/l DMF). For higher values, dilute 1/2 final colored solution with Reagent and repeat reading multiplying the final result by 2.
c) Recovery: a recovery between 95 and 99.6% was obtained adding known quantities of fructosamine to different samples.
d) Analytical sensitivity: based on an instrument minimal reading of 0.001 O.D. minimum detectable change in concentration under those conditions will be of approximately 35 umol/l.

PARAMETERS FOR AUTOANALYZERS

For programming instructions check the user's manual of the autoanalyzer in use.

WIENER LAB PROVIDES

- 2 x 50 ml (Cat. Nr. 1400050).
- 4 x 20 ml (Cat. Nr. 1009281).
- 4 x 20 ml (Cat. Nr. 1009381).
- 4 x 20 ml (Cat. Nr. 1009615).

REFERENCES

- Ambruster, D.A. - Clin. Chem 33/12:2153 (1987).
- Baker, J.R. - Clin. Chem. 31/9:1550 (1985).
- Scheicher, E.D. and Vogt, B.W. - Clin. Chem. 36/1:136 (1990).
- Young, D.S. - "Effects of Drugs on Clinical Laboratory Tests", AACC Press, 4th ed., 2001.

Symbols

The following symbols are used in 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:

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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