FRUCTOSAMINE
Instruction for use

Ref.: 97

Intended use . System for fructosamine determination by fixed-time kinetic method in serum samples.

Professional use.

[For in vitro diagnostic use.]

Test principle . Glucose binds to amino groups of proteins yielding a Schiff's base (aldimine) that after a molecular rearrange, transforms to a stable ketoamine generally known as fructosamine[1].

In alkaline pH fructosamine is converted to an enolic form that reduces nitro blue tetrazolium to a "purple formazan". The absorbance difference, after incubation in 10 minutes and 15 minutes, is proportional to fructosamine concentration in the sample[2]. The system calibration is performed with bovine matrix calibrator, calibrated with glycated polylysine. The results are presented as micromoles/liter (μmol/L).

Summary . Fructosamine determination, yielded from the bind of glucose and plasmatic proteins, is based on its reduction ability in alkaline medium. Other reducer agents may be present in serum sample and interferes in the assay. Labtest incorporated uricase and a clarifier agent based on detergents in the Fructosamine system in order to minimize the interferences of uric acid and lipemia.

The assay calibration with the glycated albumin calibrator in vitro, calibrated by glycated polylysine containing glucose labeled with ^{14}C, makes that small variations of test condition do not importantly interfere[2]. The system benefits are more consistent results with more reproducibility and accuracy.

The system uses a Work Reagent stable for 30 days, if stored at 2 - 8 ºC. The system also allows preparing the volume of the Work Reagent needed to one measure of the Fructosamine concentration.

It is easily applied to most automatic equipments which are able to measure, in a kinetic method, absorbances difference at 530 nm[5].

Methodology . NBT reduction.

Reagents

1. R 1 - Reagent 1 - Store at 2 - 8 ºC.
Reagent label bears expiration date. Buffer pH 7.3 (83 mmol/L); nitro blue tetrazolium (NBT) (967 μmol/L); uricase (≥5000 U/L); sodium azide (14.6 mmol/L) stabilizer and surfactants.

2. R 2 - Reagent 2 - Store at 2 - 8 ºC.
Reagent label bears expiration date. Buffer pH 10.4 (625 mmol/L); sodium azide (14.6 mmol/L).

3. CAL - Calibrator - Check the calibrator concentration on the bottle label. Store at 2 - 8 ºC.
Lyophilized reagent. Reagent label bears expiration date. Glycated Bovine albumin, Buffer pH 7.4 (50 mmol/L); and sodium azide (14.6 mmol/L).

Stability . Unopened reagents, when stored at indicated temperature, are stable up to expiration date shown on the label. During handling, the reagents are subject to chemical and microbial contamination that may lead to reduced stability.

Precautions and warnings

Disposal of all waste material should be in accordance with local guidelines.

The usual security cares should be applied on the reagent handling. Do not pipette the reagent with mouth.

The reagents contain sodium azide as preservative. Avoid ingestion. In case of eyes contact, immediately flush eyes with plenty of water and get medical assistance.

Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide accumulation.

Temperature and incubation time controls during the measurement must be rigorous. 1 ºC difference in the temperature produces 5% error, while 1 minute difference during the ΔA measurement produces a 20% error.

Deterioration . The Working Reagent is an alkaline solution (pH= 10.3) and, as such, is instable when expose to ambient atmosphere. In order to conserve its performance, the Working Reagent must be opened only the time needed to get the volume desired. Keep the bottle tightly closed.

Materials required not provided

1. A constant temperature water bath (37 ºC).
2. Photometer capable of measuring absorbance at 510 - 550 nm.
3. Pipettes to measure reagents and samples.
4. Timer

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Specimen collection and preparation

Use serum or plasma (EDTA and heparin) without hemolysis.
The analite is reportedly stable for about 7 days at 2 - 8 ºC and 3 months at -20 ºC.

A Standard Operating Procedure (POP) should be established to establish appropriate procedures for the collection, preparation and storage of the sample. We emphasize that the errors due to the sample may be much larger than the errors that occurred during the analytical procedure.

No known test method can offer complete assurance that human blood samples will not transmit infectious diseases. Therefore, all blood derivatives should be considered potentially infectious.

Interference

Bilirubin up to 8.0 mg/dL, and glucose up to 1000 mg/dL do not interfere significantly.

High ascorbic acid concentrations over 3.0 mg/dL and hemoglobin concentrations over 100 mg/dL result in significant negative interference.

Triglycerides concentrations up to 1000 mg/dL and uric acid up to 14 mg/dL do not significantly interfere.

Preparing the reagents

Working reagent . Use one bottle of Reagent 1 and Reagent 2 for preparing Working Reagent. Transfer all the contents of one Reagent 2 bottle to one Reagent 1 bottle and mix by inversion.

The Working Reagent is stable 30 days at 2 - 8 ºC, when no chemistry or microbiological contamination occurs (See Calibration).

The Working Reagent is an alkaline solution (pH = 10.3) and as such is unstable when exposed to the ambient atmosphere. Therefore, this reagent may have its performance compromised unpredictably if kept open, either in the automatic analyzer or on the bench. To preserve performance, the working reagent should only remain open for as long as necessary to obtain the volume to be used. Store tightly closed.

Optionally, a lower volume of the Working Reagent may be prepared by using the volume proportion 3:2 of the Reagent 1 and Reagent 2, respectively.

The Working Reagent contains 50 mmol/L phosphate buffer; 250 mmol/L carbonate buffer; 530 μmol/L nitrotetrazolium blue, uricase ≥3000 U/L, detergents and stabilizers at pH 10.3.

Calibrator . Dissolve the Calibrator bottle (nº 3) contents in 2.0 mL water reagent. Wait 30 minutes. Mix by inversion. Homogenize before using. It is stable 60 days at 2 - 8 ºC and 6 months at -10 ºC.

Manual procedure

This procedure is not applied to automated and semi-automated equipments only with flow cuvette system. It is available application procedures to automated and semi-automated equipments.

See **notes 1, 2 and 3.**

Set up two tubes and proceed as follows:

	Unknown	Calibrator
Working Reagent	1.0 mL	1.0 mL

Incubate 2 minutes at 37 ºC.

Sample	0.050 mL	------
Calibrator	------	0.050 mL

Mix and incubate exactly 10 minutes in a water bath at 37 ºC. Measure the absorbance (A_1) of the Unknown and Calibrator against water at 530 nm (510 - 550). Continue the reaction for more exactly 5 minutes and determine the absorbance (A_2) of the Unknown and Calibrator against water at 530 nm (510 - 550).

Calibration . The concentration is traceable to a proteic Calibrator calibrated with glycated polylysine sample with glucose ^{14}C labeled.

Manual calibrations
Two points calibration.
Calibrator included (Ref.: 97.3).

Calibration frequency
Perform a new calibration weekly and in the following situations:
When the internal quality control indicates.
After reagent lot change.
When a new Working Reagent is used.

Automatic Systems
Blank of reagents: water or 0.85% NaCl (150 mmol/L);
Two points calibration
Calibrator included (Ref. 97.3).

Calibration frequency
Perform a new calibration weekly and in the following situations:
When the internal quality control indicates.
After reagent lot change.
When a new Working Reagent is used.

Quality control . For quality control use Qualitrol H Level 1 and Qualitrol H Level 2 or other suitable control material. The limits and control interval must be adapted to the laboratory requirements. Each laboratory should establish corrective measures to be taken if values fall outside the control limits.

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Calculations

Determine the difference of the Calibrator and Unknown absorbance: $\Delta A = A_2 - A_1$.

Fructosamine (μmol/L) = (ΔA Unknown/DA Calibrator) x Calibrator concentration

Due the great reproductive results of the assay system, it is possible to use the factor method:

Calibration factor = Calibrator concentration/ΔA Calibrator

Fructosamine (μmol/L) = ΔA Unkown x Factor

Measurement/reportable range

The measurement result is linear between 20 μmol/L and 800 μmol/L.

If fructosamine concentration exceeds 800 μmol/L, the sample must be diluted with 0.85% NaCl. Multiply the result by the appropriate dilution factor.

Expected range . Each laboratory should evaluate the transferability of the expected values to its own patient population and, if necessary, estimate its own reference interval.

For non-diabetic individuals (all ages): 205 to 285 μmol/L[3].

Performance characteristics[10]

Recovery Studies . In two samples with fructosamine concentrations of 175 and 253 μmol/L were added known quantities of fructosamine. Subsequent analyses provided recoveries around 102 %. The mean proportional systematic error was 3.5 μmol/L or 2.0 %.

Method Comparison . A group of 40 sera were assayed by the proposed method and similar technique. Serum fructosamine values ranged from 33 - 775 μmol/L. The comparisons yielded a correlation coefficient of 0.998 and regression equation was y = 1.029x - 1.346.

An extremely positive correlation was evident between the two methods, with a systematic error of 2.19%, 2.43% and 2.53%, at the decision levels of 184 μmol/L, 272 μmol/L and 347 μmol/L, respectively. As the samples were selected randomly in outpatients and hospitalized patients, it can be inferred that the method has an adequate methodological specificity.

Imprecision - Within Run

	N	Mean (μmol/L)	SD (μmol/L)	(%) CV
Sample 1	20	302	2.51	0.83
Sample 2	20	388	3.24	0.84

Imprecision - Run-to-Run

	N	Mean (μmol/L)	SD (μmol/L)	(%) CV
Sample 1	20	302	7.58	2.50
Sample 2	20	388	8.21	2.12

Analytical sensitivity . Detection limit: 5 μmol/L. The detection limit represents the lowest measurable fructosamine concentration that can be distinguished from zero. It is calculated as two standard deviations of 20 replicates of one sample without fructosamine. It was verified that the photometric detection limit (1 cm light path cuvette) was 3.2 μmol/L, what corresponds to an absorbance equal to 0.001.

Effects of matrix dilution . A sample with a value equal to 658 μmol/L was used to evaluate the system response to the matrix dilutions with 150 mmol/L NaCl (0.85%). Using a variety of dilution factors an average recovery of 101% was found.

Notes

1. The material cleaning and drying are fundamental factors to the reagent stability and to obtain correct results.

2. The deionized or distilled water in the laboratory to prepare reagents, use in the measurements and for final glass washing must have resistivity ≥1 megaohm.cm, or conductivity ≤1 microsiemens/cm and silicates concentration must be <0.1mg/L.

3. It is suggested to consult ''www.fxol.org'' In order to review physiopathological source and drugs interference in results and methodology.

References

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2. Johnson RN, Metcalf PA, Baker JR. Clin Chem Acta 127:87-95, 1983.

3. Kruse-Jarres JD, Jarausch J, Lehmann P, Vogt BW, Rietz P. Lab Med 13:245-253, 1989.

4. Lim YS, Staley MJ. Clin Chem 32:403-404, 1986.

5. Lloyd D, Marples J. Clin Chem 30:1686-1688, 1984.

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7. Westgard JO, Barry PL, Hunt MR, Groth T. Clin Chem. 1981, 27:493-501.

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9. Basques JC. Especificações da Qualidade Analítica. Labtest Diagnóstica 2005.

10. Labtest: data on file.

Presentation

Product	Reference	Contents	
Fructosamine	97-6/15	R 1	6 X 9 mL
		R 2	6 X 6 mL
		CAL	1 X 2 mL
Fructosamine Labmax 560/400	97-4/15	R 1	4 X 9 mL
		R 2	4 X 6 mL
		CAL	1 X 2 mL

*** The number of tests in automated application procedures depends on the programmed parameters.**

Application procedures using fructosamine are available for various automated instruments.

Customer information

[Warranty conditions]

Labtest Diagnóstica warrants the performance of this product under the specifications until the expiration date shown in the label since the application procedures and storage conditions, indicated on the label and in this insert, have been followed correctly.

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Símbolos utilizados com produtos diagnósticos in vitro

Símbolos usados con productos diagnósticos in vitro

Symbols used with ivd devices

	**Conteúdo suficiente para < n > testes** Contenido suficiente para < n > tests Contains sufficient for < n > tests		**Risco biológico** Riesgo biológico Biological risk
	**Data limite de utilização (aaaa-mm-dd ou mm/aaaa)** Estable hasta (aaaa-mm-dd o mm/aaaa) Use by (yyyy-mm-dd or mm/yyyy)		**Marca CE** Marcado CE CE Mark
	**Material Calibrador** Material Calibrador Calibrator Material		**Tóxico** Tóxico Poison
	**Material Calibrador** Material Calibrador Calibrator Material		**Reagente** Reactivo Reagent
	**Limite de temperatura (conservar a)** Temperatura limite (conservar a) Temperature limitation (store at)		**Fabricado por** Elaborado por Manufactured by
	**Representante Autorizado na Comunidade Europeia** Representante autorizado en la Comunidad Europea Authorized Representative in the European Community		**Número do lote** Denominación de lote Batch code
	**Consultar instruções de uso** Consultar instrucciones de uso Consult instructions for use		**Controle** Control Control
	**Número do catálogo** Número de catálogo Catalog Number		**Controle negativo** Control negativo Negative control
	**Adições ou alterações significativas** Cambios o suplementos significativos Significant additions or changes		**Controle positivo** Control positivo Positive control
	**Produto diagnóstico in vitro** Dispositivo de diagnóstico in vitro In vitro diagnostic device		**Controle** Control Control
	**Liofilizado** Liofilizado Lyophilized		**Corrosivo** Corrosivo Corrosive
	**Período após abertura** Periodo post-abertura Period after-opening		**Uso veterinário** Uso veterinario Veterinary use
	**Instalar até** Instalar hasta Install before		

Ref.: 140214

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