FRA

Fructosamine

cobas®

Substrates

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
04537939 190	Fructosamine (150 tests)	System-ID 07 3756 9	COBAS INTEGRA 400 plus COBAS INTEGRA 800
11098993 122	Precimat Fructosamine (3 x 1 mL)	System-ID 07 9171 7	
11098985 122	Precinorm Fructosamine (3 x 1 mL)	System-ID 07 9164 4	
11174118 122	Precipath Fructosamine (3 x 1 mL)	System-ID 07 9170 9	

English

System information

Test FRA, test ID 0-056.

Intended use

In vitro test for the quantitative determination of glycated proteins (fructosamine) in human serum and plasma on COBAS INTEGRA systems.

Summary[1,2,3,4]

Fructosamine represents non-enzymatic glycation attached to blood and tissue proteins. The formation of fructosamine is a two-step reaction, which is dependent on the glucose concentration. As a first step a Schiff base is formed by the reversible coupling of glucose to protein which, in a second step, is transformed by non-reversible Amadori rearrangement to the corresponding ketoamine. This ketoamine is designated as fructosamine. The formation of fructosamine increases with the level of blood glucose. Metabolization occurs within 1 to 3 weeks, corresponding to the turnover of most serum proteins. The concentration of fructosamine thus reflects the average of the continuously varying blood glucose concentrations during this period, serving as a blood glucose memory.

Fructosamine is therefore a rapid indicator of glycemia in the diagnosis and management of diabetes mellitus.

Test principle

Colorimetric test by reaction with nitroblue tetrazolium.[5,6,7]

The colorimetric test for fructosamine (glycated protein) is based on the ability of ketoamines to reduce nitroblue tetrazolium in alkaline medium. The rate of formation of formazane is directly proportional to the fructosamine concentration and is measured photometrically at 552 nm.

Measurement is made against Roche Fructosamine Calibrator which was standardized via glycated poly-L-lysine.

Reagents - working solutions

R1 Nitroblue tetrazolium: 1.2 mmol/L; uricase (microbial): ≥ 12 µkat/L; pH 7.5; non-reactive buffer; stabilizer

SR Carbonate: 1.5 mol/L; pH 10.4

R1 is in position B and SR is in position C.

Precautions and warnings

Pay attention to all precautions and warnings listed in Section 1 / Introduction of this Method Manual.

For USA: For prescription use only.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Danger

H315	Causes skin irritation.
H318	Causes serious eye damage.
H412	Harmful to aquatic life with long lasting effects.

Prevention:

P273	Avoid release to the environment.
P280	Wear eye protection/ face protection.

Response:

P305 + P351 + P338 + P310	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER or doctor/ physician.
P362 + P364	Take off contaminated clothing and wash it before reuse.

Product safety labeling primarily follows EU GHS guidance.

Contact phone: all countries: +49-621-7590, USA: 1-800-428-2336

Reagent handling

Ready for use

Storage and stability

Shelf life at 2-8 °C	See expiration date on **cobas c** pack label
COBAS INTEGRA 400 plus system	
On-board in use at 10-15 °C	8 weeks
COBAS INTEGRA 800 system	
On-board in use at 8 °C	8 weeks

Specimen collection and preparation

For specimen collection and preparation only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable:
Serum (free from hemolysis): Collect serum using standard sampling tubes.
Plasma (free from hemolysis): Li-heparin or EDTA plasma.

Collect blood by venipuncture using an evacuated tube system.

Standardized conditions for blood withdrawal are preferable.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Stability:	3 days at 15-25 °C[8]
	2 weeks at 2-8 °C[8]
	2 months at (-15)-(-25) °C[9]

Materials provided

See “Reagents – working solutions” section for reagents.

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator’s manual for analyzer-specific assay instructions.

Application for serum and plasma

COBAS INTEGRA 400 plus test definition

Measuring mode	Absorbance
Abs. calculation mode	Kinetic
Reaction mode	R1-S-SR

Reaction direction	Increase
Wavelength A/B	552/652 nm
Calc. first/last	86/98
Unit	µmol/L

Fructosamine is measured as long analysis test (duration approximately 17 minutes).

Pipetting parameters

		Diluent (H_2O)
R1	60 µL	24 µL
Sample	6 µL	12 µL
SR	12 µL	12 µL
Total volume	126 µL	

COBAS INTEGRA 800 test definition

Measuring mode	Absorbance
Abs. calculation mode	Kinetic
Reaction mode	R1-S-SR
Reaction direction	Increase
Wavelength A/B	552/659 nm
Calc. first/last	135/155
Unit	µmol/L

Fructosamine is measured as long analysis test (duration approximately 16 minutes).

Pipetting parameters

		Diluent (H_2O)
R1	60 µL	24 µL
Sample	6 µL	12 µL
SR	12 µL	12 µL
Total volume	126 µL	

Calibration

Calibrator	Precimat Fructosamine Use deionized water as zero calibrator.
Calibration mode	Linear regression
Calibration replicate	Duplicate recommended
Calibration interval	Each lot and as required following quality control procedures.

Traceability: This method has been standardized against fructose polylysine standard.

Quality control

Reference range	Precinorm Fructosamine
Pathological range	Precipath Fructosamine
Control interval	24 hours recommended
Control sequence	User defined
Control after calibration	Recommended

For quality control, use control materials as listed in the “Order information” section. In addition, other suitable control material can be used.

The control intervals and limits should be adapted to each laboratory’s individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Follow the applicable government regulations and local guidelines for quality control.

Calculation

COBAS INTEGRA analyzers automatically calculate the analyte concentration of each sample. For more details, please refer to Data Analysis in the Online Help (COBAS INTEGRA 400 plus/800 analyzers).

Limitations - interference

In hydremic states (pregnancy for instance) it may be favorable to relate fructosamine to protein using the following formula:

$$\text{Fructosamine}_{\text{corr}} = \frac{\text{measured fructosamine} \times 72}{\text{measured total protein (in g/L)}}$$

Dysproteinemic states may affect fructosamine values.[4]

Criterion: Recovery within ± 10 % of initial value.

Serum/plasma

Icterus:[10] No significant interference up to an I index of 5 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 85 µmol/L or 5 mg/dL).

Hemolysis:[10] No significant interference up to an H index of 50 (approximate hemoglobin concentration: 31 µmol/L or 50 mg/dL).

Lipemia (Intralipid):[10] No significant interference up to an L index of 2000. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Therapeutic drug interference was tested according to the recommendations of the VDGH[a)]. No interferences were found. Exception: methyldopa, calcium dobesilate, and oxytetracycline cause artificially high fructosamine values.

Physiological ascorbic acid levels do not interfere with the fructosamine test. Ascorbic acid levels higher than 227 µmol/L (4 mg/dL) interfere with the test significantly. No significant interference up to a glucose level of 45 mmol/L (810 mg/dL).

In very rare cases, gammopathy, in particular type IgM (Waldenström’s macroglobulinemia), may cause unreliable results.[11]

For diagnostic purposes, the results should always be assessed in conjunction with the patient’s medical history, clinical examination and other findings.

a) Verband der Diagnostica und Diagnostica Geräte Hersteller. Refer to section 1 / Introduction of this Method Manual for a list of drugs tested and their concentrations.

ACTION REQUIRED

Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on COBAS INTEGRA analyzers. Refer to the CLEAN Method Sheet for further instructions and for the latest version of the Extra wash cycle list.

Where required, special wash/carry-over evasion programming must be implemented prior to reporting results with this test.

Limits and ranges

Measuring range

14-1000 µmol/L

Lower detection limit

Lower detection limit of the test:
14 µmol/L

The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying 3 standard deviations above that of a zero sample (zero sample + 3 SD, repeatability, n = 30).

Expected values[6,12]

Fructosamine concentrations were determined in 555 apparently healthy subjects between the ages of 20 and 60. A reference range of 205-285 µmol/L was determined in this study for adults without diabetes. In a poorly controlled diabetic population, mean fructosamine values were reported to be 396 µmol/L (range 228-563 µmol/L). A fructosamine concentration above the established expected value is an indicator for hyperglycemia during the preceeding 1-3 weeks or longer.

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data

Representative performance data on the COBAS INTEGRA analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using human samples and controls in an internal protocol with repeatability and intermediate precision (2 aliquots per run, 2 runs per day, 20 days). The following results were obtained:

	Level 1	Level 2
Mean	181 µmol/L	450 µmol/L
CV repeatability	0.92 %	0.65 %
CV intermediate precision	2.8 %	2.5 %

Method comparison

Fructosamine values for human serum and plasma samples obtained on a COBAS INTEGRA 700 analyzer with the COBAS INTEGRA Fructosamine reagent (y) were compared with those determined using the commercially available reagents for fructosamine on a COBAS MIRA system and an alternative manufacturer's clinical chemistry system (x). Samples were measured in duplicate. Sample size (n) represents all replicates.

		COBAS MIRA system	Alternative system
Sample size	(n)	148	200
Corr. coefficient	(r)	0.993	0.995
	(r_s)	0.990	0.991
Linear regression		y = 1.06x - 10 µmol/L	y = 0.99x - 13 µmol/L
Passing/Bablok[13]		y = 1.07x - 15 µmol/L	y = 0.98x - 11 µmol/L

The sample concentrations were between 73 and 495 µmol/L.

References

1 Johnson RN, Metcalf PA, Baker JR. Fructosamine: a new approach to the estimation of serum glycosylprotein. An index of diabetic control. Clin Chim Acta 1983;127:87-95.

2 Armbruster DA. Fructosamine: structure, analysis, and clinical usefulness. Clin Chem 1987;33:2153-2163.

3 Cefalu WT, Bell-Farrow AD, Petty M, et al. Clinical validation of a second-generation fructosamine assay. Clin Chem 1991;37:1252-1256.

4 Henrichs HR, ed. European Fructosamine Workshop. Wien Klin Wochenschr Suppl 1990;180.

5 Siedel J, Vogt B, Kerscher L, et al. Serum fructosamine assay: two different color reagents compared with reference to a HPLC-procedure. Clin Chem 1988;34:1316.

6 Kruse-Jarres JD, Jarausch J, Lehmann P, et al. A new colorimetric method for the determination of fructosamine. Lab Med 1989;13:245-253.

7 Schleicher ED, Vogt BW. Standardization of serum fructosamine assays. Clin Chem 1990;36:136-139.

8 Burtis CA, Ashwood ER. Tietz Textbook of Clinical Chemistry, 3rd ed. Pa: WB Saunders Co 1999;797.

9 Koskinen P, Irjala K. Stability of Serum Fructosamine during Storage. Clin Chem 1988;34(12):2545-2546.

10 Glick MR, Ryder KW, Jackson SA. Graphical Comparisons of Interferences in Clinical Chemistry Instrumentation. Clin Chem 1986;32:470-475.

11 Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. Clin Chem Lab Med 2007;45(9):1240-1243.

12 Melzi d'Eril GV, Bosini T, Solerte SB, et al. Performance and clinical significance of the new fructosamine assay in diabetic patients. Wien Klin Wochenschr Suppl 1990;180:60-63.

13 Bablok W, Passing H, Bender R, et al. A general regression procedure for method transformation. Application of linear regression procedures for method comparison studies in clinical chemistry, Part III. J Clin Chem Clin Biochem 1988 Nov;26(11):783-790.

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard.

CONTENT	Contents of kit
→	Volume after reconstitution or mixing
GTIN	Global Trade Item Number

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Roche Diagnostics warrants that this product will meet the specifications stated in the labeling when used in accordance with such labeling and will be free from defects in material and workmanship until the expiration date printed on the label. THIS LIMITED WARRANTY IS IN LIEU OF ANY OTHER WARRANTY, EXPRESS OR IMPLIED, INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR PARTICULAR PURPOSE. IN NO EVENT SHALL ROCHE DIAGNOSTICS BE LIABLE FOR INCIDENTAL, INDIRECT, SPECIAL OR CONSEQUENTIAL DAMAGES.

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Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
www.roche.com

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