

INTENDED USE

This reagent is intended for the quantitative determination of fructosamine in human serum. For *in vitro* diagnostic use only.

CLINICAL SIGNIFICANCE

The determination of fructosamine is most commonly performed for the evaluation of glycemic control in diabetes. Fructosamine values provide an indication of glucose levels over the preceding 2-3 weeks. A higher fructosamine value indicates poorer glycemic control.^{1,2}

TEST SUMMARY

Glycated proteins are formed by a non-enzymatic reaction between glucose and protein in which unstable Schiff bases are formed, followed by an Amadori conversion to form stable ketoamines.³ These glycated proteins include glycohemoglobin, glycoalbumin and glycated total protein. Fructosamine is a term that has come into acceptance and refers to both glycoalbumin and glycated total protein.⁴ As the average life span of these proteins is about 2-3 weeks, the level of fructosamine provides a reflection of the average glucose concentration over that time.⁵

Fructosamine and glycohemoglobin are both used to monitor diabetic control. However, each assay provides information for a specific time frame that is related to the analyte being measured. Since the life span of hemoglobin is closer to 6-8 weeks, glycohemoglobin measurements reflect the average glucose concentration over this longer period of time.⁵ Therefore, in comparison to glycohemoglobin determinations, fructosamine provides an index of intermediate-term diabetic control as opposed to the longer term for glycohemoglobin. Also, because of the shorter life span of the glycated albumin and total proteins, fructosamine measurements are more sensitive to changes in diabetic control. This provides a means to alert the physician to improvement, or deterioration in control much earlier than glycohemoglobin determinations.⁶

There have been several methods developed for the determination of fructosamine. These methods include phenylhydrazine, furosine, affinity chromatography and several colorimetric procedures.⁷ A procedure using furosine and HPLC is accepted as the reference method however, a colorimetric procedure using nitroblue tetrazolium (NBT) has gained popularity due to its speed, reproducibility and ease of automation.⁸ The reagent presented here is a modification of the commonly used NBT method.

PRINCIPLE

The fructosamine reagent set is based on the ability of ketoamines to reduce NBT to a formazan dye under alkaline conditions. The rate of formazan formation, measured at 550 nm, is directly proportional to the fructosamine concentration.

REAGENTS

Fructosamine Buffer: Carbonate buffer 100mM, pH 10.35 ± 0.1, sodium azide 0.1%.

Fructosamine Reagent: Nitroblue tetrazolium (NBT) 0.57mM, surfactant, non-reactive stabilizers and fillers.

Fructosamine Calibrator: Pooled human serum containing buffers, stabilizers and fillers.

REAGENT PREPARATION

Reconstitute the Fructosamine Reagent with the amount of Fructosamine Buffer specified on the vial label. Swirl gently to dissolve.

The Fructosamine Calibrator is supplied as a liquid stable, serum based product. It is ready to use upon opening.

REAGENT STORAGE AND STABILITY

1. Unreconstituted buffer, reagents and calibrators are stable until the expiration date on the kit label when stored at 2-8°C.
2. Upon reconstitution, the reagent should be stored at 2-8°C for best results.
3. Reconstituted reagent is stable for 7 days if stored at room temperature (15-25°C) or 30 days if stored refrigerated (2-8°C).
4. After opening, calibrator is stable for 30 days stored at 2-8°C.

PRECAUTIONS

1. For *in vitro* diagnostic use.
2. Do not use reagents past their expiration date stated on each reagent container label.
3. Do not pipette by mouth. Avoid ingestion and contact with skin.
4. Reagents in this kit contain sodium azide (0.1%) as a preservative. Sodium azide may form explosive compounds in metal drain lines. When disposing of reagents through plumbing fixtures, flush with copious amounts of water.
5. All specimens, calibrators and controls should be handled as potentially infectious, using safe laboratory procedures. (NCCLS M29-T2)⁹
6. Human serum was used in the manufacture of the calibrator. Each donor unit was tested and found negative, or non-reactive for HbsAg, HCV and HIV.

SPECIMEN COLLECTION AND STORAGE

1. Human serum, separated from the cells as quickly as possible, is the specimen of choice.
2. Collect specimens per NCCLS document H4-A3.¹⁰
3. Avoid hemolysis or contamination of the sample with hemoglobin as glycated hemoglobin will react in the same manner as fructosamine.
4. Serum specimens are stable for one week if stored at 2-8°C. Storage at -20°C is not recommended.¹¹

INTERFERENCES

1. All interference studies were performed according to the procedures recommended in NCCLS guideline No. EP7-P for interference testing in clinical chemistry.¹²
2. Bilirubin to 20 mg/dl has been demonstrated to have a negligible effect (<5%) on fructosamine results using this method.
3. Hemoglobin to 200 mg/dl has been demonstrated to have a negligible effect (<5%) on fructosamine results using this method.
4. Glucose to 600 mg/dl has been demonstrated to have a negligible effect on fructosamine results using this method.
5. See Young, et al for other interfering substances.¹³

MATERIALS PROVIDED

1. Fructosamine Buffer and Reagent.
2. Fructosamine Calibrator.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Pipetting devices.
2. Test tubes/rack.
3. Timing device.
4. Heating Block
5. Spectrophotometer capable of reading at 550nm.
6. Fructosamine Controls, catalog number F7546-CTL.

Fructosamine Reagent Set

TEST PROCEDURE (AUTOMATED-GENERAL)

Wavelength:	550nm
Assay Type:	Kinetic with Standard
Sample/Reagent Ratio:	1:21
Reaction Direction:	Increasing
Temperature:	37°C
Lag Time:	600 seconds
Read Time:	300 seconds
Reference Range:	1.61-2.68 mmol/L

TEST PROCEDURE (MANUAL)

1. Label tubes "Standard", "Control", "Sample", etc.
2. Pipette 1.0 ml reagent into all tubes and prewarm at 37°C for five minutes.
3. Add 0.05ml (50ul) of specimen to each respective tube at timed intervals.
4. After exactly ten (10) minutes at 37°C read the absorbance of each tube at 550nm (A₁). Return each tube to 37°C.
5. After exactly five (5) more minutes at 37°C, read tubes again at 550nm (A₂).
6. To determine results see "Calculations".

LIMITATIONS

1. The procedure described is linear to 8.5 mmol/L. Samples with values exceeding 8.5 mmol/L should be diluted 1:1 with saline, re-assayed, and the result multiplied by two.
2. Hemoglobin greater than 200 mg/dl may give falsely elevated results.

CALCULATIONS

A = Absorbance

$$\frac{A_2 \text{ Sample} - A_1 \text{ Sample}}{A_2 \text{ Calibrator} - A_1 \text{ Calibrator}} \times \text{Conc. of Calibrator} = \text{Fructosamine in Sample}$$

Example: if A₁ Sample = 0.100 and A₂ Sample = 0.600,
A₁ Calibrator = 0.100 and A₂ Calibrator = 0.400,
and Concentration of Calibrator = 3.0 mmol/L then:

$$\frac{0.600 - 0.100}{0.400 - 0.100} \times 3.0 \text{ mmol/L} = 5.0 \text{ mmol/L}$$

CALIBRATION

This assay requires the use of a fructosamine calibrator. The calibrator included in the kit is recommended. The use of a fructosamine calibrator from another source may produce inaccurate results. The procedure should be calibrated according to the instrument manufacturer's calibration instructions. If control results are found to be out of range, the procedure should be recalibrated.

QUALITY CONTROL

Serum controls with known normal and abnormal fructosamine values should be run routinely to monitor the validity of the reaction. These controls should be run at least with every working shift in which fructosamine determinations are performed. It is strongly recommended that each laboratory establish their own frequency of control determination.

EXPECTED VALUES

1.61 – 2.68 mmol/L¹⁴

It is strongly recommended that each laboratory establish its own normal range.

REAGENT PERFORMANCE

Assay Range: 1.0 – 8.5 mmol/L. Assay ranges for other analyzers may vary and must be validated.

Correlation: Results obtained with this reagent (y), in 45 samples ranging in fructosamine from 1.17-5.94 mmol/L, were compared with those obtained in the same samples using a reagent (x) based on the same methodology. The correlation coefficient was 0.988 and the regression equation was $y = 0.88x + 0.28$ (Std Err of Y Est = 0.19).

Precision: Precision studies were performed following a modification of the procedure contained in NCCLS document EP5-T2.¹⁵

Within Day (n=20)			Day to Day (n=20)		
Mean	S.D.	C.V.%	Mean	S.D.	C.V.%
1.97	0.04	2.0	1.91	0.06	3.1
5.57	0.10	1.8	5.72	0.14	2.4

Sensitivity: An investigation of the absorbance change per minute for ten replicates of two samples, with known concentrations of fructosamine, indicated that an absorbance change per minute of 0.042 was approximately equivalent to 1 mmol/L Fructosamine.

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