Fructosamine (plasma, serum)

1 Name and description of analyte

1.1 Name of analyte
Fructosamine

1.2 Alternative names
None, although glycated proteins are related

1.3 NLMC code
To follow

1.4 Description of analyte
Fructosamine is the name given to ketoamine products formed from the non-enzymatic attachment of a carbohydrate to a protein. The reaction between glucose and plasma proteins forms unstable Schiff bases that are converted to stable ketoamine products (fructosamine). In the context of fructosamine as a test analyte, the process usually refers to the attachment of glucose to plasma proteins.

1.5 Function of analyte
Fructosamine has no known biological function.

2 Sample requirements and precautions

2.1 Medium in which measured
Serum or plasma

2.2 Precautions re sampling, handling etc.
EDTA or lithium heparin plasma or serum samples should be separated within 3 h. Samples are stable at 2–8 ˚C for 2 weeks and at -20 ˚C for 5 weeks. When fructosamine measurements are used for monitoring, the same sample type should be used throughout (see section 4.5).

3 Summary of clinical uses and limitations of measurements

3.1 Uses
Plasma [fructosamine] is proportional to the mean blood [glucose] of an individual over the previous 1–3 weeks and has been advocated as a tool for the assessment of glycaemic control in patients with diabetes mellitus. As the mean half-life of plasma proteins is approximately 2–3 weeks, fructosamine provides a shorter term representation of glycaemic control than HbA_{1c}. There are limited and conflicting data in the literature on both the correlation with HbA_{1c} and with clinical outcomes in diabetes. No major clinical trial that focuses on developing the complications of diabetes as a clinical outcome has compared fructosamine with HbA_{1c}. It has been advocated as an alternative to HbA_{1c} measurement when the latter is likely to be an inappropriate or inaccurate measurement of mean [glucose] in an individual (e.g in a patient with a short red cell life span, see 6.1). NICE Guidelines indicate that that fructosamine should not be used as a replacement for HbA_{1c} in the general diabetic population.

3.2 Limitations

Measurements may be invalid when there are significant abnormalities of plasma protein concentrations e.g. in nephrotic syndrome, liver cirrhosis, paraproteinaemias ,during an acute phase response and in untreated thyroid disease

4 Analytical considerations

4.1 Analytical methods

The most frequently used commercially available assays are colorimetric nitroblue tetrazolium (NBT) assay and enzymatic assays. The majority of UK laboratories using the UK NEQAS scheme employ the NBT assay,

1. Colorimetric method using nitrobluetetrazolium (NBT)
Serum is added to carbonate buffer containing NBT (pH 10.8, 37 ˚C). The assay is based on the reducing properties of fructosamine under alkaline conditions. Fructosamine reduces NBT and the change in absorbance is measured at 530 nm.

2. Colorimetric method using 2-thiobarbituric acid (TBA)
Serum is heated with oxalic or acetic acid at 100 ^{o}C for 18—24 h to form 5-hydroxymethylfurfuraldehyde (HMF); protein is precipitated with trichloroacetic acid; HMF in the supernatant is heated wtih TBA at 40 ˚C for 30 min to form a derivative measured at 443 nm.

3. Colorimetric method using phenylhydrazine
Phenylhydrazine reacts with fructosamine to form a phenylhydrazone adduct with absorption at 350 nm. The absorbance is directly proportional to [fructosamine].

4. Enzymatic assay
The reactions are:
Glycated protein → (proteinase K, EC 3.4 21.64) → glycated protein fragments
Glycated protein fragments → (ketoamine oxidase, EC not assigned) → amino acids + H_20_2
H_20_2 + chromogens → (horseradish peroxidase, EC 1.11.1.7) → colour + H_20

5. Other methods have been developed but are not commercially available.

4.2 Reference method

High pressure liquid chromatography (HPLC)
Fructosamine is hydrolysed with 6M HCl at 95 ˚C for 18 h producing lysine (50%), furosine (30%) and pyridosine (10%). Furosine is quantified by HPLC using a reverse phase column with UV detection at 254 nm and 280 nm.

4.3 Reference materials

An approved reference material is not currently available for fructosamine measurement. The Randox Laboratories Ltd enzymatic method uses a calibrator assigned relative to human serum glycated with ^{14}C-glucose.

4.4 Interfering substances

The NBT method has been widely automated but interferences vary between manufacturer's methods. The manufacturer's test insert should be consulted for more details, but the following are widely reported to interfere with this assay. Note that the Roche 'second generation' assay is less prone to some interferences than its predecessor.

1. EDTA and heparin plasma samples give lower fructosamine results than serum samples in the NBT colorimetric assay.
2. Urate and glutathione produce artificially high results in the NBT assay.
3. Vitamin C >227 μmol/L interfere significantly with the NBT colorimetric assay.
4. Cysteine, methyldopa, dobesilate calcium and oxytetracycline can cause artificially low fructosamine results (all assays).
5. Bilirubin >34.2 μmol/L has been shown to cause falsely elevated fructosamine results (all assays).
6. Haemolysis can also cause falsely low results (all assays).

4.5 Sources of error

1. Colorimetric assays are affected by changes in ambient temperature.
2. There is inconsistent evidence on the magnitude of any effect that abnormalities in plasma albumin or total protein concentrations have on fructosamine values although marked abnormalities are generally regarded as a potential source of error.
3. EDTA plasma samples have been demonstrated to give a 6% negative bias relative to serum samples; for this reason, the same sample type should always be used for monitoring individual patients.

5 Reference intervals and variance

5.1.1 Reference interval (adults)

For adults without diabetes, a reference range of 205–285 μmol/L has been determined in 555 apparently healthy subjects using the NBT method. A range of 122–236 μmol/L has been established in 466 non-diabetic adults aged 20–60 y for the enzymatic method.

In patients with diabetes, specific targets are used and [fructosamine] are monitored over time to assess changes.

5.1.2 Reference intervals (others)

Pregnancy: in a study of 516 non-diabetic pregnant women, a significant reduction in [fructosamine] was recorded in successive trimesters of pregnancy; the differences were significant between Caucasian and Asian women:

	Fructosamine (μmol/L)		
	Trimester 1	Trimester 2	Trimester 3
Caucasians	n = 100	n = 100	n = 67
Range	188–256	176–252	160–219
Asian	n = 59	n = 100	n = 90
Range	180–247	176–240	171–221

5.1.3 Extent of variation

5.1.3.1 Inter-individual CV: 8.38% (NBT colorimetric method, based on data corrected for serum albumin of 40 g/L

5.1.3.2 Intraindividual CV: 5.81% (as 5.1.3.1)
5.1.3.3 Index of individuality: 0.69
5.1.3.4 CV of method
Inter-assay % CV from assay manufacturer:
Roche Cobas Integra NBT colorimetric assay: 2.8% (181 µmol/L) ; 2.5% (450 µmol/L)
Randox enzymatic assay: 1.53% (174 µmol/L); 0.83% (440 µmol/L)
5.1.3.5 Critical difference
Roche Cobas Integra NBT colorimetric method: 17.9%
Randox enzymatic assay: 16.7%
5.1.4 Sources of variation
1. Because serum [fructosamine] is proportional to blood [glucose], any changes in glycaemic control over a period of 2–3 weeks, whether due to changes in lifestyle or treatment, will influence fructosamine results.
2. Large increases or decreases in plasma [protein] will also result in variability in fructosamine results and give an inaccurate impression of glcaemic control.

6 Clinical uses of measurement and interpretation of results

6.1 Uses and interpretation
Fructosamine monitoring may be useful when it is important to monitor changes in glycaemic control over a shorter term, e.g. in pregnancy. The test can also be used as an alternative marker of glycaemia where HbA_{1c} may be less reliable as a measure of glycaemic control, for example as is the case with some assays in patients with haemoglobinopathies, silent haemoglobin variants or anaemia. However, a glycated haemoglobin method based on affinity chromatography (Trinity (Primus) HPLC) is minimally affected by most variant haemoglobins. The method has the advantage that it provides a measurement that is calibrated to produce results that are equivalent to HbA_{1c} in both units and numerical value.

6.2 Confounding factors
Fructosamine is not a suitable measure of glycaemic control in patients with rapidly changing plasma protein concentrations or when albumin turnover is increased e.g. in hyperthyroidism

7 Causes of abnormal results

7.1 High values
7.1.1 Causes:
- diabetes mellitus
- interferences (see section 4.4).

7.1.2 Investigation
Not applicable. Fructosamine should only be measured as an index of glycaemic control in patients with diabetes.

7.2. Low values
7.2.1 Causes:
- long periods of hypoglycaemia
- assay interference from certain drugs or haemolysis (see section 4.4); use of EDTA plasma
- protein losing states e.g. nephrotic syndrome, malnutrition, burns.

7.2.2 Investigation
Should an unexpectedly low value be found, investigations should be directed at the causes listed above. Hypoglycaemia may be apparent from the history.

7.3 Notes
None

8 Performance

8.1 Sensitivity, specificity etc. for individual conditions
Not applicable: fructosamine is not recommended for the diagnosis of diabetes**.**

9 Systematic reviews and guidelines

9.1 Systematic reviews

Armbruster DA. Fructosamine: Structure, Analysis and Clinical Usefulness. Clin Chem 1987; 332153-2163 *The author reviews analytical methods for fructosamine and clinical applications of its measurement. He concludes that fructosamine can be used to improve glycaemic control by responding more quickly to changes in therapy, both individually and in conjunction with* HbA_{1c}.

9.2 Guidelines
1. NICE Guideline CG15: Type 1 diabetes in children, young people and adults. July 2004, updated March 2010 and April 2010. http://guidance.nice.org.uk/CG15/Guidance (accessed 9.viii.2012)
2. NICE Guideline CG87 Type 2 diabetes: The management of type 2 diabetes May 2009 http://guidance.nice.org.uk/CG87/Guidance (accessed 9.viii.2012)
These NICE guidelines recommend that fructosamine can be used as an estimator of blood glucose control where HbA_{1c} *is contraindicated, e.g. disturbed erythrocyte turnover or abnormal haemoglobin type. Quality controlled plasma glucose profiles and total glycated haemoglobin are also recommended as alternatives (NICE CG87 section 1.3.5)*

9.3 Recommendations
See 9.2

10 Links

10.1 Related analytes
Measurement of HbA_{1c} (a glycated adduct of haemoglobin) is to be preferred for longer term monitoring of glycaemic control (over 6—8 week period

10.2 Related tests
Glucose

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