Fructosamine

Kinetic colourimetric determination of fructosamine in serum and plasma

REAGENT 1a: 8 x 6 mL
REAGENT 1b (powder): 8 x 6 mL
CALIBRATOR: 1 x 1 mL

STANDARD/CALIBRATOR: the term refers to the standard / the calibrator
REAGENT: the term refers to the single reagent
CONTROL: the term refers to the control

**CAUTION: potentially biohazardous material**
Human based serum from selected donors was used in the manufacturing of this product. Each donor unit was tested with licensed reagents and found non-reactive for HBsAg, HCV, HIV1 and HIV2 antibodies. Because no test method can offer complete assurance that human blood derived products will not transmit infectious agents, it is recommended that this product be handled with the same precautions used for patient specimens.

**PRECAUTIONS IN USE**
In addition to the possible risks regarding the reactive components, product may contain non-reactive components such as preservatives (i.e. sodium azide or other) and detergents. The total concentration of these components is lower than the limits reported by the 67/548/EEC and 1999/45/EC directives and modifications and amendments regarding classification, labelling and packaging of dangerous preparations (reagents) have been made accordingly. However, it is recommended that this product be handled carefully, that ingestion and contact with eyes, skin and mucous membranes be avoided and that laboratory reagents are used according to good laboratory practice.

**SUMMARY**
Fructosamine is a glycated protein and a time-averaged indicator of blood glucose levels that is used to assess the glycemic status of diabetics.

**PRINCIPLE**
Fructosamine, in its ketoaminic form, reduces in an alkaline medium the nitroblue tetrazolium (NBT) to formazan. The reaction rate, photometrically measured at 550 nm, is directly proportional to the concentration of the fructosamine present in the examined sample.

**REAGENTS**
Reagents, stored at 2-8 °C in unopened vials, are stable up to the expiry date indicated on the package.
Reagents must be limpid; do not use if turbid.

Components of the kit and initial concentration of reactive components in the reagent solution:
- **REAGENT 1a**
- **REAGENT 1b** powder
- **CALIBRATOR** lyophilized
  the fructosamine concentration (µmol/L) is indicated on the label of the vial.

Concentrations referred to Solution R1 - see preparation of reagent solutions: carbonate buffer 210 mmol/L pH ≥ 10.0, nitroblue tetrazolium 0.52 mmol/L, uricase ≥ 2500 U/L, sodium azide < 0.1 %

Barcode and bottle code number, if printed on reagent labels, are referred to the use of the product on Hitachi 911/912 analyzers. Please refer to the application and detailed information available upon request.

**NOTES AND LIMITATIONS**
1) **REAGENTS PECULIAR INFORMATION:**
   - the CALIBRATOR value was standardized against glycated poly-L-lysine and 14C-glucose standard.
   - a slight coloration of Solution R1 and a possible presence of suspended micro-particles will not affect the reagent performance.
2) the method determines a heterogeneous group of glycated proteins, which reactivity with the NBT compound is not completely known. The test results will therefore have to be evaluated in conjunction with other diagnostic tests results and with the patient's clinical history.

**PREPARATION OF REAGENT SOLUTIONS**
Solution R1: transfer the contents of REAGENT 1a into the REAGENT 1b vial. Mix until complete dissolution and use 10 minutes after reconstitution. Stability: 15 days at 2-8 °C, if contamination is avoided and the vial is recapped immediately after use.

Calibrator Solution: gently remove the stopper avoiding the loss of lyophilized material and add exactly 1 mL of distilled water. Replace stopper and gently swirl. Let to stand for at least 30 minutes. Gently invert to assure homogeneity of the solution.

The Calibrator, stored at 2-8 °C in an unopened vial, is stable up to the expiry date indicated on the package. The Calibrator Solution is stable 30 days at 2-8 °C if contamination is avoided and the vial is recapped immediately after use, or 3 months at -20 °C if aliquoted in small volumes. Each aliquote should be thawed only once.

**QUALITY CONTROL**
It is recommended to use following control materials to verify test accuracy:
- **Fructosamine Ctrl 1**  
  REF 16352 4x1 mL
- **Fructosamine Ctrl 2**  
  REF 16353 4x1 mL
For use, follow the instructions contained in the kit.

**SAMPLE**
Serum or plasma (heparin or EDTA) not haemolyzed. Collect samples in accordance with the NCCLS procedure reported in bibliography (1).

Stability of the sample: 14 days at 2-8 °C.
Instrumentation and materials required but not provided

- Usual laboratory equipment
- Filters photometer or spectrophotometer

ANALYTICAL PROCEDURE

Wavelength: 550 (540-560) nm
Pathlength: 1 cm
Temperature: 37 °C
Sample/Solution R1: 1/20
Reaction: fixed time (increase)

Allow reagents to reach working temperature before using. A proportional variation of the reaction volumes indicated in the analytical procedure does not change the result.

<table>
<thead>
<tr>
<th>Put into cuvette</th>
<th>Reagent Blank</th>
<th>Sample</th>
<th>Calibrator</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>0.05 mL</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sample</td>
<td>-</td>
<td>0.05 mL</td>
<td>-</td>
</tr>
<tr>
<td>Calibrator Solution</td>
<td>-</td>
<td>-</td>
<td>0.05 mL</td>
</tr>
<tr>
<td>Solution R1</td>
<td>1.0 mL</td>
<td>1.0 mL</td>
<td>1.0 mL</td>
</tr>
</tbody>
</table>

Mix carefully and incubate at working temperature for exactly 9 minutes. Read initial absorbance (A1) against distilled water and simultaneously start stop-watch. Repeat the absorbance reading after 1 minute (A2). Calculate the \( \Delta A/\text{min} \) found for Reagent Blank, Calibrator and Sample.

CALCULATION

Calculate the net \( \Delta A \) (A2 - A1) for Reagent Blank, Sample and Calibrator:

\[
[\Delta A \text{ Sample} - \Delta A \text{ Reagent Blank}] / [\Delta A \text{ Calibrator} - \Delta A \text{ Reagent Blank}] \times [\text{CAL}^*] = \mu\text{mol fructosamine} / \text{L of the sample.}
\]

[\text{CAL}^*] = concentration of fructosamine (\( \mu\text{mol/L} \)) of the CALIBRATOR

REFERENCE VALUES

Not diabetic subjects: \( \leq 285 \mu\text{mol/L} \)

It is recommended that each laboratory establish its own expected range.

PERFORMANCES

(determined on Hitachi automatic analyzer)

Interferences: the test is not affected by the presence of bilirubin up to 5 mg/dL, hemoglobin up to 0.6 g/dL, ascorbic acid up to 4 mg/dL, and triglycerides up to 1000 mg/dL. It is not necessary to test samples from fasting patients since glucose does not interfere up to a 900 mg/dL concentration.

Measuring range: 15 - 1000 \( \mu\text{mol/L} \). Samples with concentration higher than 1000 \( \mu\text{mol/L} \) must be diluted 1:10 with normal saline and result multiplied by 10.

Intra-Assay Precision: it was determined on 20 replicates of each control (2 levels - L1/L2). Results were as follows: L1: average 183.70 \( \mu\text{mol/L} \), SD 3.16, CV% 1.72 / L2: average 482.90 \( \mu\text{mol/L} \), SD 2.63, CV% 0.55.

Inter-Assay Precision: it was determined for 10 days on 2 replicates of each control (2 levels - L1/L2). Results were as follows:

<table>
<thead>
<tr>
<th>Mean</th>
<th>Within run</th>
<th>Run to run</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SD</td>
<td>CV%</td>
<td>SD</td>
</tr>
<tr>
<td>L1</td>
<td>183.60</td>
<td>2.61</td>
<td>1.42</td>
</tr>
<tr>
<td>L2</td>
<td>482.90</td>
<td>2.05</td>
<td>0.42</td>
</tr>
</tbody>
</table>

Sensitivity: 15 \( \mu\text{mol/L} \). The sensitivity was calculated on 10 replicates of normal saline and reported as the "mean zero value \( \pm 3 SD \)."

Accuracy: this test (y) was compared with a commercially available method (x). Results were as follows: N = 60, \( r = 0.99904 \), y = 0.98281 x + 5.1851

WASTE MANAGEMENT

Reagents must be disposed of in accordance with local regulations.

BIBLIOGRAPHY


Note: changes in comparison to the previous version are indicated by a vertical bar in the text margin.