**Quantitative determination of fructosamine**

**IVD**

Store at 2-8°C

**PRINCIPLE OF THE METHOD**

Under alkaline conditions the fructosamine or glycated serum proteins reduce a nitro-blue tetrazolium chloride (NBT) salt. The colour developed is directly proportional to the serum fructosamine concentration¹.

**CLINICAL SIGNIFICANCE**

Glucose forms stable glycated serum proteins with several plasmatic proteins, mainly, albumin, in covulant equation. The determination of fructosamina is based on the measurement of these glycoproteins.

The measurement of fructosamine has utility to know retrospectively (2-3 weeks) the level of glucose concentration in blood.

This test is used for control and monitoring of diabetic patients and not for diagnosis²-⁶. Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

**REAGENTS**

<table>
<thead>
<tr>
<th>Buffer</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>R 1 Carbonate</td>
<td>200 mmol/L</td>
</tr>
<tr>
<td>R 2 Enzymes</td>
<td>Nitrotrazolium chloride (NBT) 0.48 mmol/mL, Uricase 3000 U/L</td>
</tr>
</tbody>
</table>

**FRUCTOSAMINE CAL** | Calibrator lyophilised serum

**PREPARATION**

- **Working reagent (WR):**
  Dissolve (→) 1 tablet of R 2 Enzymes with one vial R 1 Buffer.
  Cap vial and mix gently to dissolve contents.
  The reagent is stable after reconstitution 15 days at 2-8ºC or 5 days at room temperature (15-25ºC). Protected from light.

- **FRUCTOSAMINE CAL:**
  Dissolve (→) the contents of one vial Calibrator with 1 mL of distilled water. Cap vial and mix gently to dissolve contents.
  The reconstituted calibrator is stable 15 days at 2-8ºC or 2 months at -20ºC.

**STORAGE AND STABILITY**

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8ºC, protected from light and contaminations prevented during their use. Do not use the tablets if appears broken.

Do not use reagents over the expiration date.

**Signs of reagent deterioration:**

- Presence of particles and turbidity.
- Blank absorbance (A) at 520 ≥ 0.30.

**ADDITIONAL EQUIPMENT**

- Spectrophotometer or colorimeter measuring at 520 nm.
- Matched cuvettes 1.0 cm light path.
- General laboratory equipment.

**SAMPLES**

Serum¹².

Don't use haemolized samples. Separated from cells as rapidly as possible. Stability of the sample: 7 days at 2-8ºC.

**PROCEDURE**

1. **Assay conditions:**
   - Wavelength: 520 (490-550) nm
   - Cuvette: 1 cm light path
   - Temperature: 37ºC
2. Adjust the instrument to zero with distilled water.
3. Pipette into a cuvette:
   - Blank
   - Calibrator
   - Sample

WR (mL) | 1.0 | 1.0 | 1.0 |
Calibrator (µL) | 100 | 100 |
Sample (µL) | 100 | 100 |

4. Mix, incubate at 37ºC and start stopwatch.
5. Read the absorbance (Aₓ) of the calibrator and sample exactly after 10 min and after 15 min (Aᵧ) of the sample addition against distilled water.

**CALCULATIONS**

\[
\frac{(\Delta A)_{\text{Sample}}}{(\Delta A)_{\text{Calibrator}}} = \frac{\text{Sample (µmol/L)}}{\text{Calibrator conc. (µmol/L)}}
\]

**QUALITY CONTROL**

Control sera are recommended to monitor the performance of assay procedures: SPINTROL H Normal and Pathologic (Ref. 1002210).

If control values are found outside the defined range, check the instrument, reagents and calibrator for problems.

Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

**REFERENCE VALUES¹**

In nondiabetic samples: 187 – 287 µmol/L¹. These values are for orientation purpose; each laboratory should establish its own reference range.

**PERFORMANCE CHARACTERISTICS**

**Measuring range:** From detection limit of 1 µmol/L to linearity limit of 1000 µmol/L.

If the results obtained were greater than linearity dilute, sample 1/2 with NaCl 9 g/L and multiply the result by 2.

**Precision:**

<table>
<thead>
<tr>
<th></th>
<th>Intra-assay (n=20)</th>
<th>Inter-assay (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (µmol/L)</td>
<td>217</td>
<td>197</td>
</tr>
<tr>
<td>SD</td>
<td>4.71</td>
<td>8.31</td>
</tr>
<tr>
<td>CV (%)</td>
<td>2.17</td>
<td>1.41</td>
</tr>
<tr>
<td></td>
<td>1.85</td>
<td></td>
</tr>
</tbody>
</table>

**Sensitivity:** 1 µmol/L = 0.0020 A.

**Accuracy:** Results obtained using SPINREACT reagents (y) did not show systematic differences when compared with other commercial reagents (x).

The results obtained using 50 samples were the following:

Correlation coefficient (r): 0.992

Regression equation: \(y = 0.991x + 1.473\)

The results of the performance characteristics depend on the analyzer used.

**INTERFERENCES**

Do not interfere: Hemolysis up to 5 g/L, bilirubin up to 20 mg/dL and triglycerides up to 6 gr/L⁻¹⁻². A list of drugs and other interfering substances with fructosamine determination has been reported by Young et. al³⁴.

**NOTES**

SPINREACT has instruction sheets for several automatic analyzers. Instructions for many of them are available on request.

**BIBLIOGRAPHY**


**PACKAGING**

Ref: 1001158