Urea is the final result of the metabolism of proteins: It is formed in the liver from their destruction.

It can appear the urea elevated in blood (uremia) in: diets with excess of proteins, renal diseases, heart failure, gastrointestinal hemorrhage, dehydration or renal obstruction.

Urea is the final result of the metabolism of proteins; It is formed in the liver from their destruction. CLINICAL SIGNIFICANCE

Urea in the sample is hydrolyzed enzymatically into ammonia (NH₄⁺) and carbon dioxide (CO₂).

Ammonia ions formed reacts with α-ketoglutarate in a reaction catalysed by glutamate dehydrogenase (GLDH) with simultaneous oxidation of NADH to NAD⁺:

\[
\text{Urea} + \text{H}_2\text{O} + 2 \text{ H}^+ \rightarrow (\text{NH}_4^+) + \text{CO}_2
\]

\[
\text{NH}_4^+ + \text{Ketoglutarate} + \text{NADH} \stackrel{\text{GLDH}}{\rightarrow} \text{H}_2\text{O} + \text{NAD}^+ + \text{L-Glutamate}
\]

The decrease in concentration of NADH, is proportional to urea concentration in the sample!

Urea aqueous primary standard 50 mg/dL

Enzymes

Buffer concentration in the sample

The decrease in concentration of NADH, is proportional to urea concentration in the sample!

General laboratory equipment:

- Matched cuvettes 1.0 cm light path.
- General laboratory equipment [Note 1].

PROCEDURE

1. Assay conditions:
   - Wavelength: 340 nm
   - Temperature: 37°C / 15-25°C

2. Adjust the instrument to zero with distilled water.
3. Pipette into a cuvette:
   - Sample
   - Standard
   - Blank

4. Mix and read the absorbance after 30 s (A₁) and 90 s (A₂).

CALCULATIONS

\[
\text{Mean (mg/dL)} \times 0.1665 = \text{mmol/L}.
\]

QUALITY CONTROL

Control Sera are recommended to monitor the performance of assay procedures.

If control values are found outside the defined range, check the instrument, reagent and calibration for problems. Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES

- Serum or heparinized plasma: Do not use ammonium salts or fluoride as anticoagulants.

Urea is stable at 2-8°C for 5 days.

PERFORMANCE CHARACTERISTICS

Measuring range: From detection limit 1 mg/dL to linearity limit 350 mg/dL.

If the concentration is greater than linearity limit dilute 1:2 the sample with CH₂O 9 g/L and multiply the result by 2.

Precision:

<table>
<thead>
<tr>
<th>Mean (mg/dL)</th>
<th>Intra-assay (n=10)</th>
<th>Inter-assay (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>40.6</td>
<td>1.22, 1.03</td>
<td>2.12, 1.15</td>
</tr>
<tr>
<td>42.5</td>
<td>0.99</td>
<td>4.99, 0.81</td>
</tr>
</tbody>
</table>

Sensitivity: 1 mg/dL = 0.00087 A.

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

The results obtained using 50 samples was the following:

Regression equation y = 0.9993x + 0.0394.

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

INTERFERENCES

It is recommended to use heparin as anticoagulant. Do not use ammonium salts or fluoride.

A list of drugs and other interfering substances with urea determination has been reported by Young et al. [1].

NOTES

1. Glassware and distilled water must be free of ammonia and ammonium salts.
2. Calibration with the aqueous standard may cause a systematic error in automatic procedures. In these cases, it is recommended to use a serum Calibrator.
3. Use clean disposable pipette tips for its dispensation.
4. BSM has instruction sheets for several automatic analyzers. Instructions for many of them are available on request.

BIBLIOGRAPHY