**INDICATION**
Uric acid determination is used for the diagnosis of gout, nitrogen retention, for the monitoring of nephropathies and it is used in all cytolytic therapies.

**METHOD PRINCIPLE**
Uric acid is oxidized by uricase into allantoin with production of hydrogen peroxide which, under the catalytic influence of peroxidase, reacts with 4-aminofenazone and N-ethyl-N-(hydroxi-3-sulphopropil)-p-toluidine (ESPT) to form a blue-violet colour:

\[
\text{Uricase} \\
\text{Uric acid + H}_2\text{O + O}_2 \rightarrow \text{Allantoin + CO}_2 + \text{H}_2\text{O}_2 \\
\text{POD} \\
\text{ESPT + 4-Aminophenazone + 2 H}_2\text{O}_2 \rightarrow \text{Indamine + 3 H}_2\text{O}
\]

The colour intensity, measured at 550 nm, is proportional to the uric acid present in the sample. The presence of ascorbate oxidase avoids interferences by ascorbic acid and other reducing agents.

**COMPOSITION**

**REAGENT A:**
- Borate Buffer pH 7.0 180 mmol/l
- Uricase > 50 U/l
- Cholesterol esterase (CHE) > 300 U/l
- 4-aminophenazone 0.25 mmol/l
- ESPT 1 mmol/l
- Peroxidase (POD) > 100 U/l
- NaN₃ < 0.095 g/l

**STANDARD:**
- 1 x 5 ml (liquid)
- Uric Acid 6 mg/dl

Verified against NIST reference material.

**PREPARATION**
Reagents are ready to use.

**STORAGE AND STABILITY**
Store at 2-8 °C. Do not freeze the reagents! The reagents are stable up to the expiry date stated on the label if contamination and evaporation are avoided, protected from light.

The above conditions are valid if the vials are opened just for the time to take the reagent, closed immediately with their cap and stored at the indicated conservation temperature.

**ANCILLARY EQUIPMENT**
- Automatic pipettes
- Photometer
- Analysis cuvettes (optical path = 1 cm)
- Temperature controlled water bath
- NaCl solution 9 g/l

**SAMPLES**
Serum, heparin or EDTA plasma, urine 24h diluted 1:10 with distilled water.

**Stability:**

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Serum/plasma</th>
<th>Urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>20-25 °C</td>
<td>3 days</td>
<td>4 days</td>
</tr>
<tr>
<td>4-8 °C</td>
<td>7 days</td>
<td>-</td>
</tr>
<tr>
<td>-20 °C</td>
<td>6 months</td>
<td>-</td>
</tr>
</tbody>
</table>

**Specimen collection / Preanalytical factors**
It is recommended that specimen collection should be carried out in accordance with NCCLS Document H11-A3.

**INTERNAL QUALITY CONTROL**
It is recommended to use controls with known uric acid concentration. Check that the values obtained are within the reference range provided.

**ANALYTICAL PROCEDURE**
Allow the reagents to reach working temperature before using.

**Calculation of results**

**Serum, plasma:**

\[
\text{Uric Acid (mg/dl)} = \frac{A \text{ sample}}{A \text{ standard}} \times 6
\]

**Urine:**

\[
\text{Uric Acid (mg/24h)} = \frac{A \text{ sample}}{A \text{ standard}} \times 600 \times l/24h
\]

**Conversion factor**
Uric Acid (mg/dl) x 59.48 = Uric Acid (µmol/l)
Uric Acid (mg/dl) x 0.05948 = Uric Acid (mmol/l)

**REFERENCE VALUES**

<table>
<thead>
<tr>
<th>Plasma/Serum</th>
<th>Female</th>
<th>Male</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/dl (µmol/l)</td>
<td>mg/dl (µmol/l)</td>
</tr>
<tr>
<td>Adults</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.3-6.1 (137-363)</td>
<td>3.6-8.2 (214-468)</td>
</tr>
<tr>
<td>Children</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-5 days</td>
<td>1.9-7.9 (113-470)</td>
<td>1.9-7.9 (113-470)</td>
</tr>
<tr>
<td>1-4 years</td>
<td>1.7-5.1 (101-303)</td>
<td>2.2-5.7 (131-470)</td>
</tr>
<tr>
<td>5-11 years</td>
<td>3.0-6.4 (178-381)</td>
<td>3.0-6.4 (178-381)</td>
</tr>
<tr>
<td>12-14 years</td>
<td>3.2-6.1 (190-381)</td>
<td>3.2-7.4 (190-440)</td>
</tr>
<tr>
<td>15-17 years</td>
<td>3.2-6.4 (190-381)</td>
<td>4.5-8.1 (190-440)</td>
</tr>
<tr>
<td>Urine</td>
<td>≤ 800 mg/24h (4.76 mmol/l)</td>
<td>balanced diet</td>
</tr>
<tr>
<td>≤ 600 mg/24h (5.57 mmol/l)</td>
<td>low purine diet</td>
<td></td>
</tr>
</tbody>
</table>

Each laboratory should establish reference ranges for its own patients population.
ANALYTICAL PERFORMANCES

Precision
Within-run and between-run coefficients of variation have been calculated on replicates of three controls at different uric acid concentration. The obtained results are reported in the following table:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Within-run</th>
<th></th>
<th>Between-run</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (mg/dl)</td>
<td>SD</td>
<td>%CV</td>
<td>SD</td>
</tr>
<tr>
<td>Serum 1</td>
<td>4.6</td>
<td>0.08</td>
<td>1.8</td>
<td>0.17</td>
</tr>
<tr>
<td>Serum 2</td>
<td>12.1</td>
<td>0.22</td>
<td>1.8</td>
<td>0.53</td>
</tr>
</tbody>
</table>

Linearity
The assay is linear up to 20 mg/dl (1190 µmol/l).

Sensitivity
Test sensitivity, in terms of limit of detection, is 0.3 mg/dl (17.84 µmol/l).

Correlation
A correlation study comparing the present method with a commercial one gave the following results:

\[ y = 1.1974x - 0.4471 \text{ mg/dl} \quad r = 0.9947 \]

Interferences
- Bilirubin > 20 mg/dl
- Hemoglobin > 50 mg/dl
- Triglycerides > 2000 mg/dl
- Ascorbic acid > 30 mg/dl

PRECAUTIONS IN USE
The reagents contain inactive components such as preservatives (Sodium azide or others), surfactants etc. The total concentrations of these components is lower than the limits reported by 67/548/EEC and 88/379/EEC directives about classification, packaging and labelling of dangerous substances. However, the reagents should be handled with caution, avoiding swallowing and contact with skin, eyes and mucous membranes. The use of laboratory reagents according to good laboratory practice is recommended.

Waste Management
Please refer to local legal requirements.

BIBLIOGRAPHY