Uric Acid PAP Fluid 5+1

ENZYMATIC IN VITRO TEST FOR THE QUANTITATIVE DETERMINATION OF URIC ACID IN HUMAN SERUM AND PLASMA.

<table>
<thead>
<tr>
<th>REF</th>
<th>88 07 96</th>
<th>12x20ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>88 03 04</td>
<td>6x100ml</td>
</tr>
</tbody>
</table>

CALIBRATION
Uric acid PAP standard (6mg/dl) 5ml 88 13 30

QUALITY CONTROL
Control Serum N 6x5ml 88 41 48B
Control Serum P 6x5ml 88 46 85B

The control intervals and limits must be adapted to the individual laboratory and country-specific requirements. Values obtained should fall within established limits. Each laboratory should establish corrective measures to be taken if values fall outside the limits.

SUMMARY
Uric acid is the final product of purine metabolism in the human organism. Uric acid measurements are used in the diagnosis and treatment of numerous renal and metabolic disorders, including renal failure, gout, leukaemia, psoriasis, starvation or other wasting conditions, and of patients receiving cytotoxic drugs.

The oxidation of uric acid provides for two approaches to the quantitative determination of this purine metabolite. One approach is the reduction of phosphotungstic acid in an alkaline solution to tungsten blue, which is measured photometrically. The method is, however, subject to interferences from drugs and reducing substances other than uric acid.

A second approach, described by Praetorius and Poulson, utilizes the enzyme uricase to oxidize uric acid; this method eliminates the interferences intrinsic to chemical oxidation. Uricase can be employed in methods that involve the UV measurement of the consumption of uric acid or in combination with other enzymes to provide a colorimetric method.

The assay described here is a modification of the colorimetric method described above. The modifications were described by Siedel. This reaction, the peroxide reacts in the presence of peroxidase, DHBSA and aminoantipyrine to form a quinoneimine dye. The intensity of the red color is proportional to the uric acid concentration and is determined photometrically.

TEST PRINCIPLE
Kinetic colorimetric assay
• Sample and addition of R1 (buffer/enzyme/DHBSA)
• Addition of R2 (buffer/enzymes/aminoantipyrine) and start of reaction

Uric acid + 2H₂O + O₂ → Allantoin + CO₂ + H₂O₂
Uricase cleaves uric acid to form allantoin and hydrogen peroxide.

2H₂O₂ + H⁺ + DHBSA + Aminoantipyrine → Quinone – diimine dye + 4H₂O
The increase in absorbance is measured.

LIMITATIONS - INTERFERENCE
Criterion: Recovery within ±10% of initial values

Icterus: No significant interference up to an index I of 40 (approx. conjugated and unconjugated bilirubin concentration 40 mg/dl).

Haemolysis: No significant interference up to an H Index of 1000 (approx. haemoglobin conc.: 1000 mg/dl).

Lipemia (Intralipid): No significant interference up to an L index of 1000 (approximate triglycerides concentration: 2000 mg/dl). There is poor correlation between turbidity and triglycerides concentration. Elevated levels of ascorbic acid produce false low values.

Uricase reacts specifically with uric acid. Other purine derivatives can inhibit the uric acid reaction.
MEASURING/REPORTABLE RANGE

Unit conversion:
mg/dl x 59.5 = µmol/l
mg/dl x 0.059 = mmol/l

Determine samples with uric acid concentrations > 25.0 mg/dl via the rerun function.
On instruments without rerun function, dilute the samples manually with 0.9% NaCl or distilled/deionized water (e.g. 1 + 1). Multiply the result by the appropriate dilution factor (e.g. factor 2).

EXPECTED VALUES

Serum/plasma:
Male: 3.4 – 7.0 mg/dl (202.3 – 416.5 µmol/l)
Female: 2.4 – 5.7 mg/dl (142.8 – 339.2 µmol/l)

Urine: (Reference range according to Krieg and Colombo)
Morning urine 37 – 92 mg/dl (2200 – 5475 µmol/l)
24 hour urine 200 – 1000 mg/24h (1200 – 5900 µmol/l/24h)
corresponding to 13 – 67 mg/dl* (773 – 3986 µmol/l*)
*Calculated from a urine volume of 1.5 l/24h

Urine (Reference range according to Tietz)
Average diet: 250 – 750 mg/24h
Low purine diet: Male: < 480 mg/24h
Female: < 400 mg/24h
High purine diet: < 1000 mg/24h

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference range. For diagnostic purposes the uric acid results should always be assayed in conjunction with the patient's medical history, clinical examinations and other findings.

ANALYTICAL SENSITIVITY (LOWER DETECTION LIMIT)

Detection limit: 0.2 mg/dl (11.9 µmol/l)
The lower detection limit represents the lowest measurable uric acid concentration that can be distinguished from zero.

Imprecision

Serum
Reproducibility was determined using human samples and controls within run (n = 20). The following results were obtained:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean mg/gl</th>
<th>SD mg/dl</th>
<th>CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>6.0</td>
<td>0.04</td>
<td>0.8</td>
</tr>
<tr>
<td>Sample 2</td>
<td>4.23</td>
<td>0.03</td>
<td>0.7</td>
</tr>
<tr>
<td>Sample 3</td>
<td>11.49</td>
<td>0.06</td>
<td>0.5</td>
</tr>
</tbody>
</table>

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<thead>
<tr>
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<th>Mean mg/gl</th>
<th>SD mg/dl</th>
<th>CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>7.21</td>
<td>0.12</td>
<td>1.7</td>
</tr>
<tr>
<td>Sample 2</td>
<td>4.43</td>
<td>0.04</td>
<td>0.9</td>
</tr>
<tr>
<td>Sample 3</td>
<td>11.03</td>
<td>0.13</td>
<td>1.2</td>
</tr>
</tbody>
</table>

METHOD COMPARISON

A comparison of the mti-diagnostics Uric Acid LS (y) with a commercial obtainable assay (x) gave with 44 samples the following result:
y = 1.008 x + 0.0645  r = 0.995

REAGENT CONCENTRATION

R1:
Phosphate buffer pH 7.4 50 mmol/l
DHBSA* 7 mmol/l
Preservative

* 3,5-Dichloro-2-hydroxy-benzenesulfonic acid
R2:
Uricase 6 kU/l
POD 5 kU/l
Aminoantipyrine 1 mmol/l
Preservative

PREPARATION AND STABILITY

For reagent start
R1: ready for use
R2: ready for use
The reagents are stable up to the expiry date in the label when stored at +2°C to +8°C.
Onboard stability: R1 28 days; Protect from light R2 28 days; Protect from light

For substrate start
Mix 5 volumes of reagent R1 with the corresponding 1 volume of enzyme reagent/R2.
Gently swirl until completely mixed. DO NOT SHAKE. This working reagent is stable (protected from light!):
3 days at +20°C to +25°C
14 days at +2°C to +8°C

SPECIMEN
Serum/plasma
Collect serum using standard sampling tubes.
Heparin, or EDTA-plasma; Stability: 5 days at +2°C to +8°C
6 moths at –20°C

Urine
Collect urine without using preservatives.
Stability: Assay urinary uric acid as soon as possible. Do not refrigerate.
Dilute urine samples manually with distilled water or 0.9% NaCl (e.g. 1 + 10). Multiply the result by the appropriate dilution factor (e.g. 11). Centrifuge samples containing precipitate before performing the assay.

TESTING PROCEDURE

Materials provided
• Working solutions as described above
Additional materials required
• Calibrators and controls as indicated below
• 0.9% NaCl

Manual procedure for substrate start:

<table>
<thead>
<tr>
<th></th>
<th>Blank</th>
<th>Standard</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard</td>
<td>---</td>
<td>20 µl</td>
<td>---</td>
</tr>
<tr>
<td>Sample</td>
<td>---</td>
<td>---</td>
<td>20 µl</td>
</tr>
<tr>
<td>Working reagent</td>
<td>1000 µl</td>
<td>1000 µl</td>
<td>1000 µl</td>
</tr>
</tbody>
</table>

Mix R1 and R2 in a ratio 5+1. Gently swirl until completely mixed.

Mix, incubate for 5 min at +37°C or 10 min at +20°C or +25°C. Read absorbance of the sample against reagent blank within 30 min.

Calculation: by standard

\[
\frac{\Delta A_{\text{Sample}}}{\Delta A_{\text{Standard}}} \times \text{standard conc. (6mg/dl)} = \text{Uric acid conc. (mg/dl)}
\]
**Manual procedure for reagent start:**

<table>
<thead>
<tr>
<th>Wavelength</th>
<th>Hg 546 nm (490-550)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>+25°C / +30°C / +37°C</td>
</tr>
<tr>
<td>Cuvette</td>
<td>1cm light path</td>
</tr>
<tr>
<td>Zero adjustment</td>
<td>against reagent blank</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
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<th><strong>Standard</strong></th>
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<tbody>
<tr>
<td>Standard</td>
<td>---</td>
<td>20 µl</td>
<td>---</td>
</tr>
<tr>
<td>Sample</td>
<td>---</td>
<td>---</td>
<td>20 µl</td>
</tr>
<tr>
<td>R1</td>
<td>1000 µl</td>
<td>1000 µl</td>
<td>1000 µl</td>
</tr>
</tbody>
</table>

Mix and incubate 60 seconds at assay temperature, read initial absorbance, then add:

<table>
<thead>
<tr>
<th></th>
<th><strong>R2</strong></th>
<th><strong>R2</strong></th>
<th><strong>R2</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>200 µl</td>
<td>200 µl</td>
<td>200 µl</td>
</tr>
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</table>

Mix, start stopwatch simultaneously. Repeat reading after exactly 4 minutes.

**Calculation:** by standard

\[
\frac{\Delta A_{\text{Sample}}}{\Delta A_{\text{Standard}}} \times \text{standard conc. (6mg/dl)} = \text{Uric acid conc. (mg/dl)}
\]

**DISPOSAL**

Please note the legal regulations.

**NOTES**

For in vitro diagnostic use.

Exercise the normal precautions required for handling all laboratory reagents.

**LITERATURE**