URIC ACID (Uricase – PAP) Enzymatic Determination of Serum Uric Acid

INTENDED USE
NS Biotec uric acid reagent is intended for the in vitro quantitative determination of uric acid in serum, plasma and urine on both automated and manual systems.

CLINICAL SIGNIFICANCE
Uric acid is the major end product of purine metabolism and is one of the components of the non-protein nitrogen fraction in plasma. Most uric acid formation occurs in the liver and is derived either from ingested or endogenous nucleoproteins. Approximately, half of the total uric acid in the body is eliminated daily by urinary excretion and destruction in the intestinal tract. Numerous disease states and physiological conditions are associated with alterations in serum uric acid concentrations. Serum uric acid levels are characteristically elevated in gout and disorders involving either uric acid synthesis or excretion. Other common etiologies of hyperuricaemia include renal dysfunction, leukemia, polythyroidism, ketoacidosis, glucose-6-phosphate deficiency and Lesch-Nyhan syndrome. Decreased uric acid levels have been described in renal tubular absorption defects, Hodgkin’s disease, bronchogenic carcinoma, severe hepatocellular disease, and xanthinuria.

ASSAY PRINCIPLE
The most commonly used techniques for uric acid determinations are based on the reduction of phosphotungstate by uric acid in alkaline medium. These methods required serum deproteinization and are subject to interference by numerous substances which are present in serum. More recently, techniques have been developed which use uricase to improve specificity. These methods are either direct ultraviolet spectrophotometric, or dye coupled techniques.

NS Biotec uric acid reagent is based on the dyes coupled technique and combines the use uricase enzyme with the peroxidase/3,5-dichloro-2-hydroxybenzene sulfonic acid/4-aminoantipyrine system for the measurement of uric acid in human serum.

The series of reactions involved in the assay system are as follows:
1. Uric acid is converted by uricase into allantoin and hydrogen peroxide.
2. In presence of peroxidase (POD), the formed hydrogen peroxide formed affects the oxidative coupling of 3,5-dichloro-2-hydroxybenzene sulfonic acid (DHBS) and 4-aminoantipyrine (4-AAP) to form a red-colored quinoneimine dye.

Uric acid + O2 + 2 H2O2 → Allantoin + CO2 + H2O2
2 H2O2 + 4-AAP + 3,5 DHBS → Quinoneimine dye + 2H2O

The intensity of the color produced is directly proportional to uric acid concentration. It is determined by measuring the increase in absorbance.

EXPECTED VALUES

<table>
<thead>
<tr>
<th>Serum or plasma</th>
<th>Child: 2.0 – 5.5 mg/dl</th>
<th>Adult Male: 3.4 – 7.0 mg/dl</th>
<th>Adult Female: 2.4 – 6.0 mg/dl</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>119 – 327 mol/l</td>
<td>202 – 416 mol/l</td>
<td>143 – 357 mol/l</td>
</tr>
</tbody>
</table>

| Urine  | 0.25 – 0.75 g/day  | 1.48 – 4.43 mmol/day  |

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 assessed in conjunction with the patient’s medical history, clinical examination, and other findings.

REAGENTS

| R1  | Uric acid standard 6.0 mg/dl  |
| R2  | Phosphate buffer, pH 7.0 50 mmol/l  |
|     | 3,5-DHBS 4.0 mmol/l  |
|     | Uricase >400 U/l  |
|     | Peroxidase >1000 U/l  |
|     | Ascorbate oxidase 1500 U/l  |
|     | 4-Aminoantipyrine 0.3 mmol/l  |

- **Reagent Preparation & Stability**
  All reagents are ready for use and stable up to the expiry date given on label when stored at 2–8°C.

- **SPECIMEN**
  Serum, plasma and urine.
  * The only accepted anticoagulants are heparin and EDTA.

- **Specimen Preparation & Stability**
  - **For serum specimen**
    No special preparation of the specimen is necessary. The best specimen is unhemolysed serum, EDTA or heparinized plasma, and should be analyzed on the day of collection. When stored at 4°C, specimens are stable for 3 to 5 days; specimens are stable at ~20°C for at least 6 months.
  - **For urine specimen**
    For 24 hours collections add 15 ml sodium hydroxide to the container before collection to keep urine alkaline, pH should be checked. If the pH is less than 8.0, it should be adjusted by sodium hydroxide. Uric acid in urine is stable for 3 to 5 days at room temperature. Don’t refrigerate urine specimens. Urine specimens diluted 1:10 with water prior to analysis.

- **PROCEDURE**
  - **Manual Procedure**
    | Wavelength | 500 - 550 nm |
    | Cuvette    | 1 cm light path |
    | Temperature| 20-25 or 37°C |
    | Zero adjustment | against reagent blank |

  Specimen: Serum or plasma
  Mix, incubate for 5 minutes at 37°C or 10 minutes at 20-25°C. Measure the absorbance of specimen (A<sub>specimen</sub>) and standard (A<sub>standard</sub>) against reagent blank.

  The color is stable for 60 minutes.
  - **Automated Procedure**
    User defined parameters for different auto analyzers are available upon request.

- **CALCULATION**
  Calculate the uric acid concentration by using the following formulae:

  Uric acid Concentration= Absorbance of Specimen× Standard value
  Absorbance of Standard

- **REFERENCE RANGE**
  - **Child**
    | Age | Value |
    |-----|-------|
    | 0-2 | 2.0 – 5.5 mg/dl  |
    | 3-12 | 3.4 – 7.0 mg/dl  |
    | 13-20 | 2.4 – 6.0 mg/dl  |

  - **Adult Male**
    | Value |
    | 3.4 – 7.0 mg/dl  |
    | 202 – 416 mol/l  |
    | 143 – 357 mol/l  |

  - **Adult Female**
    | Value |
    | 2.4 – 6.0 mg/dl  |
    | 143 – 357 mol/l  |

  - **Urine**
    | Value |
    | 0.25 – 0.75 g/day  |
    | 1.48 – 4.43 mmol/day  |

  - **Uric acid standard**
    | Value |
    | 6.0 mg/dl  |
Unit conversion

mg/dl x 59.48 = µmol/l

For urine specimen the results must be multiplied by the dilution factor and 24 hours urine volume in liters.

LINEARITY

When run as recommended, the assay is linear up to 25 mg/dl (1.49 mmol/l).

If result exceeds 25 mg/dl (1.49 mmol/l), specimen should be diluted with 0.9% NaCl solution and reassayed. Multiply the result by the dilution factor.

SENSITIVITY

The sensitivity is defined as the change of analytical response per unit change in analyte concentration at a path length of 1 cm.

When run as recommended the sensitivity of this assay is 0.2 mg/dl (11.9 µmol/l).

QUALITY CONTROL

It is recommended that controls (normal and abnormal) be included in:

- Each set of assays, or
- At least once a shift, or
- When a new bottle of reagent is used, or
- After preventive maintenance is performed or a clinical component is replaced.

Commercially available control material with established uric acid values may be routinely used for quality control.

Failure to obtain the proper range of values in the assay of control material may indicate:

- Reagent deterioration,
- Instrument malfunction, or
- Procedure errors.

The following corrective actions are recommended in such situations:

- Repeat the same controls.
- If repeated control results are outside the limits, prepare fresh control serum and repeat the test.
- If results on fresh control material still remain outside the limits, then repeat the test with fresh reagent.
- If results are still out of control, contact NS Biotic Technical Services.

INTERFERING SUBSTANCES

- Anticoagulants:
  The only acceptable anticoagulants are heparin and EDTA.

- Bilirubin:
  No interference from free bilirubin up to level of 8.0 mg/dl and from conjugated bilirubin up to a level of 12 mg/dl.

- Drugs:
  Of the drugs tested in vitro, methyldopa and noramidopyrine cause artificially low uric acid values at the tested drug level. For a more comprehensive review of drugs affecting uric acid assays refer to the publication by Young.²

- Haemoglobin:
  Haemoglobin levels higher than 5.0 g/l decrease the apparent uric acid concentration significantly.

- Lipemia:
  No significant interference.

- Others:
  Physiological ascorbic acid in serum, plasma or urine specimens do not interfere with the test. Ascorbic acid levels higher than 3.0 mg/dl in serum or plasma specimen and 25 mg/dl in urine specimen decrease the apparent uric acid concentration significantly.

WARNING & PRECAUTIONS

- NS Biotic uric acid reagent is for in vitro diagnostic use only. Normal precautions exercised in handling laboratory reagents should be followed.
- Warm up working solution to the corresponding temperature before use.
- The reagent and sample volumes may be altered proportionally to accommodate different spectrophotometer requirements.
- Valid results depend on an accurately calibrated instrument, timing, and temperature control.
- The reagent blank will not exceed an absorbance of 0.06 but don’t use the reagent if it is turbid or if the absorbance is greater than 0.2 at 500 nm.
- If specimen is grossly lipemic or icteric use serum blank by adding 20 l of patient serum to 1.0 ml distilled water and read absorbance against water blank. Subtract this absorbance from the test absorbance to correct for the lipemia or icterus samples.

BIBLIOGRAPHY