

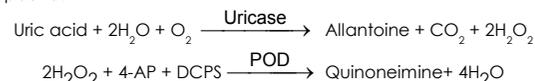
URIC ACID



Uric Acid. Uricase-POD. Liquid Ref.:URI-016
Quantitative Determination of uric acid
 Only for *in vitro* use in clinical laboratory
 Store at 2-8°C 2 x 125 mL

PRINCIPLE OF THE METHOD

Uric acid is oxidized by uricase to allantoin and hydrogen peroxide (2H₂O₂), which under the influence of POD, 4-aminophenazone (4-AP) and 2,4-Dichlorophenol sulfonate (DCPS) forms a red quinoneimine compound:



The intensity of the red color formed is proportional to the uric acid concentration in the sample^{1,2}.

CLINICAL SIGNIFICANCE

Uric acid and its salts are end products of the purine metabolism. With progressive renal insufficiency, there is retention in blood of urea, creatinine and uric acid. Elevated uric acid level may be indicative of renal insufficiency and is commonly associated with gout^{1,5,6}. Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

REAGENTS

| | | |
|----------------------|-------------------------------------|-----------|
| R 1 | Phosphate pH 7.4 | 50 mmol/L |
| Buffer | 2-4 Dichlorophenol sulfonate (DCPS) | 4 mmol/L |
| R 2 | Uricase | 60 U/L |
| Enzymes | Peroxidase (POD) | 660 U/L |
| | Ascorbate oxidase | 200 U/L |
| | 4-Aminophenazone (4-AP) | 1 mmol/L |
| URIC ACID CAL | Uric acid aqueous primary standard | 6 mg/dL |

PREPARATION

Working reagent (WR):
 Mix equal volumes of R 1 Buffer and R 2 Enzymes.
 The working reagent is stable 1 week at refrigerator (2-8°C) or 4 days at room temperature (15-25°C).

STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8°C, protected from light and contaminations prevented during their use. Do not use reagents over the expiration date.

URIC ACID CAL Once open is stable up to 1 month when stored tightly closed at 2-8°C, protected from light and contaminations prevented during their use.

Signs of reagent deterioration:

- Presence of particles and turbidity.
- Blank absorbance (A) at 520 nm \geq 0.16.

ADDITIONAL EQUIPMENT

- Spectrophotometer or colorimeter measuring at 520 nm.

- Matched cuvettes 1.0 cm light path.
- General laboratory equipment.

SAMPLES

- Serum or plasma¹: Stability 3-5 days at 2-8°C or 6 months at -20°C.
- Urine (24 h)¹: Stability 4 days at 15-25°C, pH >8. Dilute sample 1/50 in distilled water. Mix. Multiply results by 50 (dilution factor); If urine is cloudy; warm the specimen to 60°C for 10 min to dissolve precipitated urates and uric acid. Do not refrigerate.

PROCEDURE

- Assay conditions:
 Wavelength: 520 nm (490-550)
 Cuvette: 1 cm light path
 Temperature 37°C / 15-25°C
- Adjust the instrument to zero with distilled water.
- Pipette into a cuvette:

| | Blank | Standard | Sample |
|-------------------------------------|-------|----------|--------|
| WR (mL) | 1.0 | 1.0 | 1.0 |
| Standard ^(Note 1-2) (μL) | -- | 25 | -- |
| Sample (μL) | -- | -- | 25 |

- Mix and incubate for 5 min at 37°C or 10 min at 15-25°C.
- Read the absorbance (A) of the samples and Standard, against the Blank. The colour is stable for at least 30 minutes.

CALCULATIONS

Serum or plasma

$$\frac{(A) \text{ Sample}}{(A) \text{ Standard}} \times 6 (\text{Standard conc.}) = \text{mg/dL uric acid in the sample}$$

Urine 24 h

$$\frac{(A) \text{ Sample}}{(A) \text{ Standard}} \times 6 \times \text{vol. (dL) urine 24 h} = \text{mg/24 h uric acid}$$

Conversion factor: mg/dL x 59.5 = μmol/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, 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

Serum or plasma:

| | | | |
|--------|-------------------|---------|----------------------|
| Women | 2.5 - 6.8 mg/dL | \cong | 149 - 405 μmol/L |
| Men | 3.6 - 7.7 mg/dL | \cong | 214 - 458 μmol/L |
| Urine: | 250 - 750 mg/24 h | \cong | 1.49 - 4.5 mmol/24 h |

These values are for orientation purpose; each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS

Measuring range: From detection limit of 0.03 mg/dL to linearity limit of 25 mg/dL.

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

Precision:

| | Intra-assay (n=20) | | Inter-assay (n=20) | |
|-------------|--------------------|------|--------------------|--------|
| | Mean (mg/L) | SD | CV (%) | CV (%) |
| Mean (mg/L) | 4.74 | 11.4 | 4.72 | 11.2 |
| SD | 0.03 | 0.06 | 0.07 | 0.15 |
| CV (%) | 0.63 | 0.56 | 1.58 | 1.36 |

Sensitivity: 1 mg/dL = 0.0347 A.

Accuracy: Results obtained using BSM 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): 0.99.

Regression equation: y = 1.005x + 0.0005.

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

INTERFERENCES

No interferences were observed to bilirubin up to 170 μmol/L, hemoglobin up to 130 mg/dL and ascorbic acid up to 570 μmol/L².

A list of drugs and other interfering substances with uric acid determination has been reported by Young et al. ^{ab3,4}.

NOTES

- Calibration with the aqueous standard may cause a systematic error in automatic procedures. In these cases, it is recommended to use a serum Calibrator.
- Use clean disposable pipette tips for its dispensation.
- BSM has instruction sheets for several automatic analyzers. Instructions for many of them are available on request.**

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