URIC ACID (Uricase-PAP)

INTENDED USE

Vitro 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.

METHOD

Enzymatic, colorimetric method (Uricase/PAP) with uricase and 4-aminoantipyrine.
Liquid stable single reagent.

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[1]. 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[2]. Other common etiologies of hyperuricaemia include renal dysfunction, leukemia, polythyroidism, ketoacidosis, glucose-6-phosphate deficiency and Lesch-Nyhan syndrome[2-4]. Decreased uric acid levels have been described in renal tubular absorption defects, Hodgkin's disease, bronchogenic carcinoma, severe hepatocellular disease, and xanthinuria[1].

ASSAY PRINCIPLE

The most commonly used techniques for uric acid determinations are based on the reduction of phosphotungestate by uric acid in alkaline medium[5,6]. 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[7], or dye coupled techniques.[8,9] Vitro uric acid reagent is based on the dye coupled technique and combines the use uricase enzyme with the peroxidase/2,4,6 – tribromo-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 2,4,6-tribromo-benzene sulfonic acid (TBHB) and 4-aminoantipyrine (4-AAP) to form a red-colored quinoneimine dye.

$$\text{Uric acid} + O_2 + 2\,H_2O \xrightarrow{\text{Uricase}} \text{Allantoin} + CO_2 + H_2O_2$$

$$2\,H_2O_2 + \text{4-AAP} + \text{2,4,6 TBHB} \xrightarrow{\text{POD}} \text{Quinoneimine dye} + 2H_2O$$

The intensity of the color produced is directly proportional to uric acid concentration. It is determined by measuring the increase in absorbance at 500 – 550 nm.

EXPECTED VALUES

Serum or plasma[10]

Child:	2.0 – 5.5 mg/dl 119 – 327 µmol/l
Adult Male:	3.4 – 7.0 mg/dl 202 - 416 µmol/l
Adult Female:	2.4 – 6.0 mg/dl 143 - 357 µmol/l

Urine[10]

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

R_1	Uric acid standard	6.0	mg/dl
R_2	Phosphate buffer, pH 7.0	50	mmol/l
	TBHB	2.0	mmol/l
	Uricase	>400	U/l
	Peroxidase	>1000	U/l
	Ascorbate oxidase	1500	U/l
	Ferrocyanide	40	µmol/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^{0}C.

SPECIMEN

Serum is the only accepted specimen. Avoid hemolysis.

Specimen Preparation & Stability For serum specimen

Separate serum from clot/cells within 8 hours at room temperature or 48 hours at 2–8^{0}C. Uric acid is stable at 2-8 ^{0}C for 7 days. Freezing of the samples is not recommended.

PROCEDURE

- **Manual Procedure**

Wavelength	500 - 550 nm
Cuvette	1 cm light path
Temperature	20-25 or 37 ^{0}C
Zero adjustment	against reagent blank
Specimen	Serum or plasma

	Blank	Standard	Specimen
R_2	1.0 ml	1.0 ml	1.0 ml
Standard	……	20 µl	……
Specimen	……	……	20 µl

Mix, incubate for 5 minutes at 37^{0}C or 10 minutes at 20-25^{0}C. Measure the absorbance of specimen ($A_{specimen}$) and standard ($A_{standard}$) 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 =

$$\frac{\text{Absorbance of Specimen}}{\text{Absorbance of Standard}} \times \text{Standard value}$$

- **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.

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.

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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 Vitro 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[12].
- **Hemoglobin:**
  Hemoglobin 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 & PRECAUTION

- Vitro 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 µl 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.

PERFORMANCE CHARACTERISTICS

Imprecision

Reproducibility was determined using in an internal protocol. The following results were obtained.

	Within Run		Between Day	
Control	Level I	Level I	Level I	Level II
Number of samples	40	40	40	40
Mean (mg/dl)	4.7	4.6	4.6	10.45
SD (mg/dl)	0.14	0.32	0.32	0.36
CV (%)	2.3	6.8	6.8	3.4

Method Comparison

Comparison studies were carried out using a similar commercially available reagent as a reference. Serum and urine samples were assayed in parallel and the results

compared by least squares regression. The following statistics were obtained.

Number of sample pairs	45
Range of results	1.9 – 10.6 mg/dl
Mean of reference method results	5.29 mg/dl
Mean of Uric Acid results	5.65 mg/dl
Slope	0.935
Intercept	0.71 mg/dl
Correlation coefficient	0.989

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

LINEARITY

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.

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SYMBOL DECLARATION

	Manufacturer
	Consult instructions for use
LOT	Batch code (Lot #)
REF	Catalog number
	Temperature limitation
IVD	In vitro diagnostic medical device
	Use by
	Caution. Consult instructions
	Keep away from light

ORDERING INFORMATION

REF	SIZE	REF	SIZE
13901	2 x 25 ml	13908	4 x 50 ml
13902	2 x 50 ml	13909	2 X 125 ml
13903	1 x 100 ml	13910	3 X 100 ml
13904	5 X 20 ml	13911	4 X 100 ml
13905	5 X 25 ml	13912	4 X 125 ml
13906	10 X 20 ml	13913	4 X 250 ml
13907	10 X 25 ml	13914	2 X 500 ml

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