URIC ACID

URICASE METHOD

Intended Use:
Uric Acid test reagent/kits is a medical device intended for the estimation of Uric Acid in serum or plasma.

Clinical Significance
Uric acid is the end product of the purine metabolism. Uric acid is excreted to a large degree by the kidneys and to smaller degree in the intestinal tract by microbial degradation.Increased levels are found in Gout, arthritis, impaired renal functions and starvation. Decreased levels are found in Wilson's disease, Fanconis syndrome and yellow atrophy of the liver.

Principle

$$\text{Uric acid} + H_2O \xrightarrow{\text{Uricase}} \text{Allantoin} + H_2O_2$$

$$H_2O_2 + \text{4Aminoantipyrine} + \text{Phenolic Compound} \xrightarrow{\text{Peroxidase}} \text{Red Quinoneimine dye} + H_2O$$

Reagent Composition:

R1 Uric Acid Reagent	Phosphate Buffer - 85 mmol/L Uricase - 1.6 KU Peroxidase - 1.75 KU 4-Amino Antipyrine- 0.55 mmol/L Chromogen, Surfactant and Stabilizers
Standard	8mg/dl

Working Reagent Preparation
Reagent is ready to use.

Stability and Storage
Store at 2-8ºC
All the kit contents are stable until the expiry date stated on thelabel. Do not use reagents beyond the expiration date.
Store the vials tightly closed protected from light and preventcontaminations during the use.

Discard if signs of deterioration appear:
- Presence of particles and turbidity.

Materials required
- Photometer or spectrophotometer with a thermostat cellcompartment set at 25/30/37ºC, capable of reading at 520 (500-540) nm.
- Stopwatch, strip-chart recorder or printer.
- Cuvettes with 1-cm path length
- Pipettes to measure reagent and samples.

Sample and Stability
Serum or plasma
Serum or heparinized plasma free of hemolysis.
Uric Acid is reported to be stable in the sample for 3-5days when stored at 2-8^{0}C.

Assay Procedure
Pipette into clean dry test tubes labeled as Blank (B), Standard (S) and Test (T)

Addition Sequence	Blank	Std	Test
R1 Uric Acid Reagent	1000µl	1000µl	1000µl
Standard (S)	-	20µl	-
Sample	-	-	20µl

Mix and incubate for 10 minutes at 37^{0}C or at RT (30^{0}C) for 15 Min. Measure the absorbance of the Standard (Abs.S), and Test Sample (Abs.T) against the Blank at 520nm, within 30 min.

Calculation:

$$\text{Uric Acid in mg/dl} = \frac{\text{Abs of Test}}{\text{Abs of Std}} \times 8$$

Quality Control
To ensure adequate quality control (QC), each run should include aset of controls (normal and abnormal) with assayed values handledas unknowns.

If the values are found outside of the defined range, check the instrument, reagents and procedure.
Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable results

Linearity
This procedure is linear upto 30 mg/dl. If values exceed this limit, dilute the sample with normal saline(NaCl 0.9%)and repeat assay.Calculate the value using the proper dilution factor.

Reference Values
Serum/ Plasma (Males) : 3.4-7.0 mg/dl
(Females): 2.5 – 6.0 mg/dl
It is recommended that each laboratory establishes its own normal range representing its patient population.

General System Parameters

Mode	End Point
Reaction	Increasing
Wavelength	520 (500-540) nm
Blank with	Reagent
Sample Volume	20µl
Reagent Volume	1000µl
Std Conc.	8 mg/dl
Incub. Temp.	37^{0}C
Incub. Time	10min
Delay Time	5 sec
Normal Range	2.5 – 7.0 mg/dl
Linearity	Up to 30mg/dl
Unit	mg/dl

Notes
1. Reagents must be stored at 2-8°C till expiry.
2. If a larger volume of reagent is required for the absorbance reading, requisite volumes can be taken in multiples keeping the same ratio of reagent to specimen/standard.
3. The rate for colour development of Enzyme Reagent can be reduced by ensuring the storage of Reagent (highly photosensitive) at 2-8^{0}C.

Reference

1. Trinder, P., (1969) Ann. Clin. Biochem. 6:24
2. Fossati, R, Prencipe, L., (1980) Clin. Chem. 26:227.

For *in vitro* Diagnostic use only.