URIC ACID
URICASE-POD Method

DIATEK

CLINICAL SIGNIFICANCE :

Uric acid is end product of the purine metabolism. In gout, the most common complication of hyperuricemia, increased serum levels of uric acid lead to formation of monosodium urate crystals around the joints. Further causes of elevated blood concentrations of uric acid are renal diseases with decreased excretion of waste products, starvation, drug abuse and increased alcohol consume as well as use of certain medicaments. High uric acid levels also constitute a indirect risk factor for coronary heart disease. Hypouricemia is seldom observed and associated with rare hereditary metabolic disorders.

TEST PRINCIPLE :

$$\text{Uric Acid} + H_2O + O_2 \xrightarrow{\text{Uricase}} \text{Allantoin} + CO_2 + H_2O_2$$

$$2\,H_2O_2 + \text{4-Aminoantipyrine} \xrightarrow{\text{POD}} \text{Chinonimine} + 3\,H_2O + \text{TBHBA}$$

(TBHBA= 2,4,6-Tribrom3-hydroxybenzoic acid)

The pink colored is formed from 4-aminoanti- pyrine, TBHBAl, and hydrogen peroxide. The absorbance of the solution of this colordye is proportional to the concentration of Uric Acid in the sample.

REAGENTS COMPOSITION :

Reagent R1

Phosphatebuffer (pH 7.0): 100 mmol/l
TBHBA : 1 mmol/l
4-Aminoantipyrine : 0.3 mmol/l
K4[Fe(CN)6] : 10 µmol/l
Peroxidase (POD): 2 kU/l
Uricase : 30 U/l

Standard:
Standard Conc. : 6 mg/dl

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2 - 8 ºC, and contaminations prevented during their use. Do not use reagents over the expiration date. Once opened the reagent is stable for at least 1 months at 2 – 8 °C and 1 month on-board the auto analyzer at approximately10°C.

KIT CONTENTS :

CODE No. :	UA01	UA02	UA03
Pack size :	(2x25 ml),	(5x25 ml)	(5x100 ml)
Reagent-1 (R1) Uric Acid Reagent	2 x 25 ml	5 x 25 ml	5 x 100 ml
Uric Acid Standard 6 mg/dL)	1 x 1 ml,	1 x 1 ml	1 x 2 ml

SAMPLES : Serum free of hemolysis , plasma (EDTA / heparin) Serum should be separated from blood as soon as possible.
Urine sample to be diluted 1:10 (1 part + 9 part) in distilled water.

MATERIALS REQUIRED BUT NOT PROVIDED :

Uric Acid Control (Use of assayed QC sera is recommended to validate test result).

INTERFERENCES

Gross haemolysis, sample interfere with the results. Samples with elevated bilirubin (> 5mg/dl), Hemoglobin (>100 mg/dl), Intralipid (> 1000 mg/dl) and Ascorbic Acid (> 5mg/dl) may have a slight effect on accuracy.

ASSAY CONDITIONS:

Wavelength : ..... 546 nm (540 - 555 nm)
Cuvette: ..... 1 cm light path
Constant temperature 37ºC
Reaction ..... End Point
Standard Conc..... 6 mg/dL
Linearity..... 20 mg/dL
Unit..... mg/dl
Slope of Reaction ..... Increasing
Blanking..... Reagent

PROCEDURE :

Pipette into test tubes labeled Blank (B), Standard (S) and Test (T) as follows:

	B	S	T
Uric Acid Reagent (R1)	1.0 ml	1.0 ml	1.0 ml
Uric Acid Standard		20 µl	
Specimen			20 µl

Mix and incubate for 5 mint at 37ºC or 10 mint. at RT (25ºC - 30ºC). Read absorbance of Standard (S) and Test (T) against Blank (B) with 546 nm. The final color is stable for 1 hour at R.T.

CALCULATIONS :

$$\text{Uric Acid in mg/dl} = \frac{\text{Abs. of T}}{\text{Abs. of S}} \times 6$$

REFERENCE RANGE :

Men: 3.2 -7.2 mg/dL
Women: 2.4 - 6.2 mg/dL

Urine : < 800 mg/dL with normal food
< 600 mg/dL with reduced purine food

The above reference range is guideline and all the laboratories must establish their own normal reference range. Final diagnosis should be made with correlation of clinical factors.

PRECAUTIONS :

1. Storage conditions as mentioned on the kit to be adhered.
2. Use clean glassware and microtips while pipetting Uric Acid Standard. Replug Uric Acid Standard vial after use
3. Avoid contamination of the reagent during the assay process.
4. Before the assay begins, bring all the reagents to room temperature.
5. If a larger volume of reagent is required for the absorbance reading, requisite volume can be taken in multiples, keeping the same ratio of reagent to specimen/standard.
6. Do not freeze or expose the reagents to high temperature and protect from direct sunlight as it will affect the performance of the kit.
7. Programmes for specific autoanalysers are available on request.
8. For accuracy of results, the assay procedure, reagent preparation and storage has to be meticulously followed.
9. As with all the diagnostic procedures, the physician should evaluate data obtained by the use of this kit in light of other clinical information.

LINEARITY AND DETECTION LIMIT :

The assay is linear up to Uric Acid concentration of 20 mg/dl. The results of the performance characteristics depend on the analyzer used. If the results obtained were greater than linearity limit, dilute the sample 1 : 4 with Normal Saline and multiply the result by 4.

BIBLIOGRAPHY :

1. Thomas L. Clinical Laboratory Diagnostics. 1st ed. Frankfurt: TH-Books Verlagsgesellschaft; 1998. p. 208-14.

2. Newman DJ, Price CP. Renal function and nitrogen metabolites. In: Burtis CA, Ashwood ER, editors. Tietz Textbook of Clinical Chemistry. 3rd ed. Philadelphia: W.B Saunders Company; 1999. p. 1204-70.

Manufactured by : **DIATEK Healthcare Pvt. Ltd.**
P-54 , Phase-I , Kalyani Industrial Estate, Kalyani, Dist. : Nadia, Pin : 741235 E-mail : diatek@rediffmail.com
Corporate Office : P-27, Kalindi Housing Scheme, Kalindi, Kolkata- 700089, Help line : 033 - 25223075