Lifechem™ - URIC ACID - LR

(URICASE – POD METHOD WITH DHBS)

CLINICAL SIGNIFICANCE:

Uric acid circulates in the plasma as sodium urate and it is end product of purine metabolism. The determination of uric acid is useful in differentiation of gout from non-gout arthritis. Uric acid estimation in serum or plasma has a diagnostic significance in failure of kidney and gout.

Levels of uric acid is elevated in acute infectious diseases, severe uremia, and toxemia of pregnancy. Uric acid levels increase in treatment of corticosteroids, Leukemia, Polycythemia, Renal Dysfunction, Atherosclerosis, Diabetes, and Hypothyroidism or in some Genetic Diseases. Uric acid levels are decreased in Wilson's disease.

In the enzymatic method, Uricase is used along with peroxidase (POD), 4-aminoantipyrine and DHBS. The enzymatic method is very simple, specific and measures true Uric Acid.

TEST PRINCIPLE:

Enzymatic conversion of uric acid to allantoin quantitatively produces hydrogen peroxide. Hydrogen peroxide thus produced is measured through an indicator reaction involving peroxidase.

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

$$2H_2O_2 + \text{DHBS} + \text{4AAP} \xrightarrow{\text{Peroxidase}} \text{Red quinoneimine Complex} + HCl + 4H_2O$$

NORMAL RANGE:

Serum Uric Acid

Women	:	2.4 –5.7 mg/dl
Men	:	3.4 –7.0 mg/dl
Urine Uric Acid	:	250 –750 mg/24 hour's urine

KIT CONTENTS:	**Code No. KUA1 (1x50 ml)**	**Code No. KUA2 (2x50 ml)**
Reagent 1 Enzyme Reagent	50 ml	2x 50 ml
Reagent 2 Uric Acid Standard (5 mg/dl)	3 ml	3ml

SPECIMEN:

Serum, Heparinised plasma or EDTA plasma.
Urine. Urine should be diluted 1:10 with distilled water before use.

WORKING REAGENT PREPARATION:

All reagents are ready-to-use and are stable at 2-8^{0}C till the expiry date mentioned on the labels. When opened, care should be taken to avoid contamination.

PROCEDURE:

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

	B	S	T
Enzyme Reagent (1)	1.0ml	1.0ml	1.0ml
Uric Acid Standard (2) (5mg/dl)	--	0.02ml	--
Specimen	--	--	0.02ml
Mix and incubate for 10 minutes at 37^{0}C(or)15 minutes at R.T.			

Mix well and read absorbance of Standard (S) and Test (T) against Blank (B) at 520 nm or with green filter (505-520 nm).

The final colour is stable for 30 minutes at R.T.

CALCULATIONS:

Serum Uric Acid in mg/dl $= \frac{\text{Abs of T}}{\text{Abs of S}} \times 5$

Urine Uric Acid in mg/dl $= \frac{\text{Abs of T}}{\text{Abs of S}} \times 50$

QUALITY CONTROL:

It is recommended to include Assayed Quality control serum (Level 1 & II.) with each assay batch to verify the performance of the procedure. Failure to obtain the proper range of values in the assay of control sera may indicate reagent deterioration, instrument malfunction or procedural errors.

SYSTEM PARAMETERS:

Reaction Type	:	End Point	Units	:	mg/dl
Wave length	:	520 nm (505-520)	Blanking with	:	Reagent
Flow Cell temp	:	37^{0}C	Low Normal	:	2.4
Sample volume	:	20 µl	High Normal	:	7.0
Reagent volume	:	1000µl			
Standard Conc.	:	5			

LINEARITY: The Kit is linear upto 30 mg/dl

NOTES:

1. Contamination of Standard and Reagents must be avoided. After use all the reagents must be immediately stored back at 2-8^{0}C.
2. Incase of turbidity in urine, heat the sample for 10 minutes at 60^{0}C to dissolve any possible Urate precipitates.
3. Avoid use of detergents for cleaning glassware. Contamination by soap or glycerol will affect this assay.
4. Enzyme reagent may develop a slight pink colouration as the reagent ages, but it will not interfere with the test.
5. Should not freeze or expose the reagents to higher temperature it may affect the performance of the kit.

BIBLIOGRAPHY:

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