

URIC ACID

(URICASE-POD Method)





INTENDED USE:

This reagent kit is used for in-vitro quantitative determination of Uric acid in human serum, plasma and urine.

TEST PRINCIPLE:

Uric Acid Liquid Reagent kit provides a method for detecting uric acid in serum/plasma/urine. In the assay, uricase catalyzes the conversion of uric acid to allantoin, hydrogen peroxide (H₂O₂) and carbon dioxide. The H₂O₂ then, in the presence of horseradish peroxidise (HRP), reacts with 4- aminoantipyrene and DHBS to generate red quinoneimine complex, which is measured spectrophotometrically in absorbance max of 505 (505-520) nm.

REACTION:

Uric Acid+ $2H_2O+O_2 \xrightarrow{}$ Allantoin+ $CO_2+H_2O_2$ $2H_2O_2 + 4$ -AAP + DHBS \xrightarrow{POD} Red Quinineimine complex + 4H₂O + HCl

KIT CONTENTS:

Reagent 1: Uric Acid Enzyme Reagent Reagent 2: Uric Acid Standard (6 mg/dl)

Product Insert : 01 No.

PREPARATION OF THE WORKING REAGENT:

All the reagents are ready to use.

STORAGE AND STABILITY:

All the reagents should be stored in 2-8°C and are stable till the expiry date mentioned in the labels.

SPECIMEN COLLECTION AND STORAGE:

Unhemolysed serum/heparinised plasma is recommended. Urine should be diluted 1:10 with distilled water before use.

PRECAUTIONS: /

- 1. Storage conditions as mentioned on the kit to be adhered.
- Do not freeze or expose the reagents to higher temperature as it may affect the performance of the kit.
- Before the assy bring all the reagents to room temperature. 3.
- Incase of turbidity in urine, heat the sample for 10 minutes at 60°C to dissolve any possible Urate precipitates.
- 5. Avoid contamination of the reagent during assay process.
- 6. Use clean glassware free from dust or debris.

PROCEDURE (Automated):

Refer to specific instrument application instructions.

TEST PROCEDURE: | i

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

Pipette into Test Tube	Blank	Standard	Test
Uric Acid reagent	1.0 ml	1.0 ml	1.0 ml
Standard	-	25 μΙ	-
Sample	-	-	25 μΙ

Mix well and incubate for 5 minutes at 37°C.

Read absorbance of Standard (A_s), and Test (A_T) against Blank (A_R) at 505 nm or with green filter (500-540nm).

CALCULATIONS:

Serum/Plasma Uric Acid Conc. in mg/dl

$$= \frac{\text{Abs of A}_{\tau} - \text{A}_{B}}{\text{Abs of A}_{S} - \text{A}_{B}} \quad \text{X 6 (Conc. of Standard)}$$

Urine Uric Acid Conc. in mg/dl

$$= \frac{\text{Abs of A}_{T} - \text{A}_{B}}{\text{Abs of A}_{C} - \text{A}_{D}} \times 6 \times 10$$

NORMAL VALUES*:

Serum/Plasma Uric Acid

Women : 2.7-6.5 mg/dl Men : 4.0 - 7.2 mg/dl

Urine Uric acid: 250 - 750 mg/24 hrs urine

*It is recommended that each laboratory should establish its own normal range.

PERFORMANCE:

1. Linearity: 20 mg/dl

2. Comparison:

A comparison between Uric Acid Liquid Reagent (y) and a commercially available test(x) using 55 samples gave the following results:

y = 0.986x + 0.664

r = 0.99

3. Precision:

	Within Run			Run to Run		
	Mean	S.D.	C.V.%	Mean	S.D.	C.V.%
Low	5.4	0.4	0.9	5.4	0.2	2.1
High	9.5	0.4	0.4	10.0	0.35	1.8

4. Specificity: No interference upto 1gm/l Hemoglobin & Bilirubin 20 mg/dl.

CLINICAL SIGNIFICANCE:

Serum uric acid is the end product of purine metabolism in the body tissues and is cleared through the kidneys by glomerular filtration.(I). Most animals can metabolize uric acid to more readily excreted products, but humans lack the necessary

enzyme, urate oxidase (uricase), as a result of the presence of two "mutations" in the human gene for uricase.(2) Increased uric acid levels may result from leukemia, polycythemia, ingestion of foods high in nucleoproteins (e.g. liver and kidney) or impaired renal function. Gout results from the deposit of uric acid in body joints.

AUTOMATED APPLICATIONS:

Uric Acid Liquid Reagents can be used with Hitachi 700 series, RA 50, 1000 XT, Express 550, Syncron CX4, LISA 200, BTR 810/820/830, Erbachem-5, Ranlab etc. Application sheets for use on specific semiautomatic / batch analysers are available on request.

Input parameters for semiauto / auto analyzers are given below:

INPUT PARAMETERS	VALUES	
Type of reaction	Endpoint	
Wavelength	505 nm.	
Incubation time	5 minutes	
Standard concentration	6 mg/dl	
Units	mg/dl	
Temperature	37°C	
Linearity	20 mg/dl	
Reagent volume	1.0 ml	
Sample/Standard volume	25 μΙ	

QUALITY CONTROL:

For accuracy, it is necessary to run known serum controls with each assay.

REFERENCES:

- 1. ARCHIBALD RM. Colorimetric measure-ment of uric acid. Clin Chem. 1957Apr; 3(2):102-105.
- GJORUP S, POULSEN H, PRAETORIUS E. The uric acid concentration in serum de-termined by enzymatic spectrophotometry. Scand J Clin Lab Invest 1955;7(3):201 -203.
- 1. Barham, D., and Trinder, P., Analyst 97, 142-145 (1972).
- 2. Fossati, P., Presscipe, L., and Bertr, G., Ciln. Chem.

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