URIC ACID LIQUID

ENZOPAK

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(URICASE METHOD)
Reagent kit for quantitative estimation of Uric acid in serum or plasma.

BACKGROUND AND SYNOPSIS:
Uric acid is produced by the action of xanthine oxidase on xanthine and hypoxanthine, which are products of nucleic acid degradation. The chemical method of estimation of uric acid is based on the uric acid forming a blue phosphotungstate complex. However, this method is not specific as other reducing substances are present in serum or plasma which continues to produce colour by phosphotungstate complex. In 1980, Fossati et, al described a modified Trinder's method for the assay of uric acid.

ENZOPAK Uric Acid is formulated on this method with the advantage that the method is specific and sensitive.

PRINCIPLE:
Uricase is a very specific enzyme acting on Uric Acid, end products being allantoin and hydrogen peroxide. Peroxidase is used to utilize hydrogen peroxide (proportional to uric acid concentration) to convert chromogens to coloured complex. The intensity of colour produced is proportional to uric acid concentration and is measured photometrically at 546 nm (530-570) nm or with YELLOW filter).

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

$$H_2O_2 + \text{4-Aminoantipyrine} + \text{TOOS} \xrightarrow{\text{Peroxidase}} \text{coloured complex}$$

DIAGNOSTIC SIGNIFICANCE:
Uric Acid levels in any subject may vary during a day from time to time and from day to day. The changes represent the contents in diet and metabolism besides pathological condition.

The diagnostic values showing increased in levels are found in kidney failure and gout. Elevated levels are found in acute infectious diseases, severe uremia, toxemia of pregnancy, leukemia and some malignant diseases or conditions.

REAGENT COMPOSITION:

ACTIVE REAGENTS	Concentration
Reagent-1	
* Buffer	100 mmol/L
* 4 – AAP	1 mmol/L
* POD	≥ 2000 U/L
pH 7.0 ± 0.5 at 25^{0}C	
Reagent-2	
* Buffer	100 mmol/L
* TOOS	0.5 mmol/L
* Uricase	≥ 600 U/L
pH 7.0 ± 0.1 at 25^{0}C	
Uric Acid Standard (6 mg/dl)	

Also contains non-reactive fillers & stabilizers.

PRESENTATION:

	No. of Bottles	
All reagents to be stored at 2-8^{0}C	**2 x 10 ml**	**2 x 20 ml**
• 1 Uric Acid – Liquid	2 x 8 ml	2 x 16 ml
• 2 Uric Acid – Liquid	2 x 2 ml	2 x 4 ml
• Uric-Acid Standard (6 mg/dL)	1 x 2.5 ml	1 x 2.5 ml

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PRECAUTION:
ENZOPAK Uric acid is for *IN-VITRO* diagnostic use only.
Reagent Contains Sodium Azide, DO NOT INGEST.

PREPARATION OF WORKING REAGENT:
For 2 x 10 ml. & 2 x 20 ml
Carefully transfer the contents of 1 bottle of 2 URIC ACID into the bottle of 1 URIC ACID. Mix well. Wait for 2 minutes before use.

Alternatively for flexibility as much of working reagent may be made as and when desired by mixing 4 parts of 1 Uric Acid liquid and 1 part of 2 Uric Acid liquid.

REAGENT STORAGE & STABILITY:
All reagents included in the kit are stable at 2-8^{0}C until the expiry date indicated on the label. Working reagent is stable for 4 weeks at 2-8^{0}C. A slight rise in the blank reading due to stored life will not affect the test results.

SPECIMEN COLLECTION:
Fresh, clear fasting serum with no hemolysis is the specimen of choice. However, plasma collected from blood using heparin as an anticoagulant may be used.

REACTION PARAMETERS:

- Type of Reaction : End Point
- Wavelength : 546 nm (530-570 nm)
- Flowcell Temperature : 37 ^{0}C
- Incubation : 10 min. at 37^{0}C
- Std. Concentration : 6 mg/dL
- Sample Volume : 50 µl (0.05 ml)
- Reagent Volume : 1.0 ml.
- Light Path : 1.0 cm.
- Zero setting with : Reagent Blank

TEST PROCEDURE:
For instruments with 1.0 ml/0.5 ml cuvette capacity or flow cells requirements.

PIPETTE INTO TEST TUBES	Procedure for 1 ml.			Procedure for 0.5 ml.		
	BLK	STD.	TEST	BLK	STD.	TEST
• WORK. Rgt. (ml)	1.0	1.0	1.0	0.5	0.5	0.5
• STD. (ml)	-	0.05	-	-	0.025	-
• SAMPLE (ml)	-	-	0.05	-	-	0.025

Mix and incubate at 37^{0}C for 10 min. and read absorbance of test and standard against reagent blank at 546 nm (530-570 nm or YELLOW filter).

STABILITY OF FINAL COLOUR DEVELOPED:
The colour of reaction mixture is stable for one hour when protected from direct light.

TEST RESULTS:

$$\text{Uric Acid Concentration (mg/dL)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Std.}} \times 6$$

To convert Uric Acid concentration (mg/dL) to micromoles/liter uses the following equation.

Micromoles/lit. = mg/dl x 59.5

LIMITATIONS FOR INTERFERENCE:
As per studies carried out for interference. Following results were obtained.

- No Interference from Hemoglobin upto 500 mg/dL.
- No Interference from free Bilirubin upto 15 mg/dL.
- No Interference from Lipemic (Measured as Triglycerides) upto 1000 mg/dl.

NORMAL VALUES:

Male	: 3.2 - 7.0 mg/dL	(190.4 - 416.5 µmol/L)
Female	: 2.6 - 6.0 mg/dL	(154.7 - 357 µmol/L)

NORMAL VALUES (URINE):
Urine Uric acid content: 400-900 mg/24 hours.

LINEARITY:
This method is linear upto 20 mg/dL. For sample values higher than linearity limit, dilute the samples suitably with 0.9 % saline and repeat the assay. Apply proper dilution factor to calculate the test results by applying dilution factors.

REFERENCES:

TRIVEDI, R.C., REBBAR, L.BERKA, E., AND Strong, I: Clin.Chem 24:1908(1978).
FOSSATI, P., PRINCIPLE, L., BERTI, G:Clin Chem 26, 227-231 (1980).

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