Manual Procedure

Automated procedure on request

MEDICHEM MIDDLE EAST
Clinical Chemistry Reagents
Liquid Stable Reagents

Cat. No. 12631	R1	3 x 40	ml
For 180 tests	R2	3 x 20	ml
Cat. No. 12632	R1	3 x 70	ml
For 315 tests	R2	3 x 35	ml
Cat. No. 12633	R1	2 x 160	ml
For 480 tests	R2	2 x 80	ml

Uric acid PAP

Enzymatic colorimetric method

Liquid Reagents

Test Principle

$$\text{Uric Acid} + 2\,H_2O + O_2 \xrightarrow{\textit{Uricase}} \text{Allantoine} + CO_2 + H_2O_2$$

$$2\,H_2O_2 + \text{4-Aminophenzone} + \text{TOOS} \xrightarrow{\textit{Peroxidase}} \text{Red quinone} + 4\,H_2O$$

Uric acid is oxidized by uricase to allantoine and hydrogen peroxide. The oxidative condensation of TOOS and 4-aminophenazone in the presence of peroxidase and hydrogen peroxide produces a red chromogen which has an absorbance at 550 nm. The intensity of the color produced is proportional to uric acid concentration in the sample.

Concentrations in the test

Reagent R1		
Phosphate-Buffer (pH = 8.30)	100	mmol/L
4-Aminophenazone	1	mmol/L
Ascorbat Oxidase	≥ 5.0	U/ml
Peroxidase	≥ 2.9	U/ml
Detergent		
Reagent R2		
Potassium hexacyanoferrat (II)	11.0	µmol/L
Uricase	≥ 0.3	U/ml
TOOS (N-ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methylaniline)	7.0	mmol/L
Activators, stabilizer and clearer		
Standard : The concentration as indicated on vial.		

Stability and preparation of working reagent

Reagent R1: *liquid.*

Reagent R2: *liquid.*

All reagents are stable up to expiry date given on the label when stored at +2 ➔ +8 °C.

Working Reagent:

Mix 2 volumes of R1 with 1 volume R2, Avoid any foam formation.
Stability in dark: 2 weeks at 2 - 8 °C.

Note: Don't use if the reagent is turbid or contains obvious microbial growth.

Specimen collection and handling

1. Non-hemolyzed serum, heparinized, or EDTA plasma.
2. Uric acid in serum is stable for 3 days at 2 - 8 °C and 6 months at - 20 °C.
3. Collect 24 hours urine on 5 ml 12N NaOH. Dilute (1+9) with double distilled water and multiply result by 10.
4. Random (fresh) urine sample: dilute (1+9) with 0.01N NaOH and multiply result by 10.

Calibrator / Standard

Medical U Cat. No. 15011
Uric acid STD. Cat. No. 16211

Quality control

Meditrol N Cat. No. 15171
Meditrol P Cat. No. 15181

Procedure

Wavelength	Hg 546 nm (530 - 570 nm)
Spectrophotometer	550 nm
Cuvette	1 cm light path
Temperature	37°C / 20 - 25 °C
Measurement	against reagent blank
Reaction	end point

Assay

	Blank	Calibrator / Standard	Sample
Distilled water	20 µl	--	--
Calibrator / Standard	--	20 µl	--
Sample	--	--	20 µl
Working Reagent	1000 µl	1000 µl	1000 µl
Mix, incubate for 5 min. at 37°C or 10 min. at 20 – 25 °C. Read the absorbance (A). The final color is stable for 30 min.			

Calculations

Serum:

$$\text{Conc.}_{\text{Uric acid}}\,(\text{mg/dl}) = \frac{A_{\text{Sample}}}{A_{\text{Cal./STD.}}} \times \text{Conc.}_{\text{Cal./STD.}}\,(\text{mg/dl})$$

Urine:

$$\text{Uric acid}_{\text{Urine /24 hr.}} = \frac{\text{U.A mg/dl x (vol./ L) Urine /24 hr.}}{100}\ \text{g/24 hr.}$$

$$\mu\text{mol/L} \underset{59.5\ X}{\overset{X\ 0.0168}{\rightleftharpoons}} \text{mg/dl}$$

Linearity

Up to 20 mg/dl (1190 µmol/L).
If the result exceeds 20 mg/dl, repeat the test using diluted sample (1+1) with sodium chloride solution (0.9 %) and multiply the result by 2.

Interferences

1. Bilirubin up to 20 mg/dl has been demonstrated to have a negligible effect (< 5 %) on uric acid results using this method.
2. Hemoglobin up to 100 mg/dl has been demonstrated to have a negligible effect (< 5 %) on uric acid values. Hemoglobin greater than 100 mg/dl cause falsely elevated uric acid values.
3. Lipemic samples may cause falsely elevated uric acid levels.
4. Elevated ascorbic acid levels can result in falsely depressed uric acid values.
5. See Young, *et. al.,* for other interfering substances.

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Precautions

The reagent contains sodium azide (0.1 %) as a preservative. Don't ingest. Avoid skin and eye contact. Sodium azide may react with copper or lead plumbing to form explosive metal azides. Upon disposal flush with large amounts of water.

Reference range

Serum

1 – 4 wk.		< 5.2	mg/dl
2 – 12 mth.		< 6.2	mg/dl
Children		< 6.1	mg/dl
Adults	women	< 5.7	mg/dl
	men	< 7.0	mg/dl

Urine

1st morning urine	37 – 92	mg/dl
Urine / 24 hr.	0.20 – 1.00	g/24 hr.

Reference

1. Trinder, P., Am. Clin. Biochem. 6 (1969), 24.
2. Schettler, G. u. E. Nüssel, Arbeits- u. Präventivmed.10 (1975) 25.
3. Young, DS., Effects of Drugs on Clinical Laboratory Tests, fifth edition 2000, AACC Press, Washington, D.C.