URIC ACID - PAP

Colorimetric Enzymatic Method

Cat.No. 101-0324	*Size 5 x 20 ml*
Cat.No. 101-0332	*Size 4 x 50 ml*
Cat.No. 101-0212	*Size 12 x 50 ml*
Cat.No. 101-0333	*Size 6 x 100 ml*
Cat.No. 101-0211	*Size 4 x 250 ml*

PRINCIPLE:

Uric acid is converted by uricase to allantoin and H_2O_2, which under the catalytic influence of peroxidase, oxidizes 2,4-dichlorphenolsulphonate and 4-amino-antipyrine to form a red-violet quinoneimine.The intensity of the color formed is proportional to the concentration of uric acid.

$$\text{Uric acid} + 2H_2O + O_2 \xrightarrow{\text{Urikase}} \text{allantoin} + CO_2 + H_2O_2$$

$$2H_2O_2 + 4-\text{amin oantipyrine} + 2,4-\text{dichlorphenolsulphonate} \xrightarrow{\text{Peroxidase}}$$

$$\xrightarrow{\text{Peroxidase}} \text{quinoneimine} + 4H_2O$$

SAMPLE:

Serum, heparinized or EDTA plasma.
Urine diluted 1:10 with distilled water.
Stable for 5 days at +2 °C to +8 °C.

REAGENTS:

1. Buffer	
Hepes buffer, pH 7.4	50 mmol/L
2,4-dichlorphenolsulphonate	4 mmol/L
2. Enzyme reagent	
Uricase	60 U/L
Peroxidase (POD)	660 U/L
4-aminoantipyrine	1 mmol/L
Ascorbate Oxidase	200 U/L
3. Standard	
Uric acid	Standard concentration see on the vial label

Store at +2 °C to +8 °C.

PREPARATION OF REAGENTS:

Reconstitute the contents of one vial enzyme reagent (2) with the corresponding volume of buffer (1)
The working solution is stable 4 weeks at +2 °C to +8 °C or 2 weeks at +15 °C to +25 °C in the dark bottles.

PROCEDURE:

Wavelength:	520 nm (490 - 550 nm)
Cuvette:	1 cm light path
Temperature:	25 °C, 37 °C
Color stability:	30 min
Zero:	reagent blank (one reagent blank per series only)

Pipette into test tubes	Reagent blank	Standard	Sample
Standard	-	20 µl	-
Sample	-	-	20 µl
Working reagent	1000 µl	1000 µl	1000 µl
Mix and incubate for 10 min. at room temperature or 5 min. at 37 °C. Avoid exposure to direct sunlight. Measure the absorbance of the standard and sample against the reagent blank within 30 minutes.			

NOTE:

Volumes can be proportionally changed.

CALCULATION:

$$\frac{A_{\text{sample}}}{A_{\text{standard}}} \times \text{stand.conc.} = \mu\text{mol/L acid}$$

EXPECTED VALUES:

Serum	Women	142- 339 µmol/L (2.4 - 5.7 mg/dl)
	Men	202 - 416 µmol/L (3.4 - 7.0 mg/dl)
Urine		1.5 - 4.5 mmol/24h (250 - 750 mg/24h)

LINEARITY:

up to 1170 µmol/L (20 mg/dl)

QUALITY CONTROL:

CONTRO-N	20 x 5 ml	Cat. No. 101-0083
CONTRO-P	20 x 5 ml	Cat. No. 101-0084

NOTE:

1. If the uric acid concentration >1170 µmol/L, dilute sample 1:2 with physiological solution and repeat the assay (result x 2).
2. Hemoglobin concentration >2 g/L and bilirubin concentration >340 µmol/L influence the results of the uric acid.
3. Reagents contain sodium azide as stabilizer. Do not swallow. Avoid contact with the skin and mucous membrane.

REFERENCES

1. Trinder P. Ann. Clin. Biochem., 6, 24 (1996).
2. Pileggi R. and Barthelmai, W. klin. Wochenschr. 40, 585-589 (1962).