URIC ACID - L

Enzymatic colorimetric method for the quantitative determination of Uric Acid in serum, plasma and urine

CE

IVD

ORDER INFORMATION

REF	Kit size
GA4865 00	12x50 ml
KL4865 00	10x60 ml
BK4865 00	4x60 ml

INDICATION

Uric acid determination is used for the diagnosis of gout, nitrogen retention, for the monitoring of nephropathies and it is used in all cytolytic therapies.

METHOD PRINCIPLE

Uric acid is oxidized by uricase into allantoin with production of hydrogen peroxide which, under the catalytic influence of peroxidase, reacts with 4-aminofenazone and N-ethyl-N-(hydroxi-3-sulphopropil)-p-toluidine (ESPT) to form a blue-violet colour:

$$\text{Uric acid} + H_2O + O_2 \xrightarrow{\text{Uricase}} \text{Allantoine} + CO_2 + H_2O_2$$

$$\text{ESPT} + \text{4-Aminophenazone} + 2\ H_2O_2 \xrightarrow{\text{POD}} \text{Indamine} + 3\ H_2O$$

The colour intensity, measured at 550 nm, is proportional to the uric acid present in the sample.
The presence of ascorbate oxidase avoids interferences by ascorbic acid and other reducing agents.

COMPOSITION

REAGENT A:

Borate Buffer pH 7.0	180 mmol/l
Uricase	> 50 U/l
Cholesterol esterase (CHE)	> 300 U/l
4-aminophenazone	0.25 mmol/l
ESPT	1 mmol/l
Peroxidase (POD)	> 100 U/l
NaN_3	< 0.095 g/l

STANDARD:	1x5 ml
Uric Acid	6 mg/dl

Verified against NIST reference material.

Preparation
Reagents are liquids ready to use.

Storage and stability
Store at 2-8 °C. Do not freeze the reagents! The reagents are stable up to the expiry date stated on the label if contamination and evaporation are avoided, protected from light. The above conditions are valid if the vials are opened just only for the time to take the reagent, closed immediately with their cap and stored at the indicated conservation temperature.

ANCILLARY EQUIPMENT

- Automatic pipettes
- Photometer
- Analysis cuvettes (optical path = 1 cm)
- Temperature controlled water bath
- NaCl solution 9 g/l

SAMPLES

Serum, heparin or EDTA plasma, urine 24h diluted 1:10 with distilled water.
Stability:

	Temperature		
	20-25 °C	4-8 °C	- 20 °C
Serum/plasma:	3 days	7 days	6 months
Urine:	4 days	-	-

Specimen collection / Preanalytical factors
It is recommended that specimen collection should be carried out in accordance with NCCLS Document H11-A3.

INTERNAL QUALITY CONTROL

It is recommended to use controls with known uric acid concentration. Check that the values obtained are within the reference range provided.

ANALYTICAL PROCEDURE

Allow the reagents to reach working temperature before using.

Pipette into disposable or well clean cuvettes :

	Blank	Standard	Sample
Reagent A	1000 µl	1000 µl	1000 µl
Distilled H_2O	25 µl		
Standard	-	25 µl	-
Sample	-	-	25 µl
Mix and incubate for 15 minutes at room temperature (20-25 °C) or for 10 minutes at 37 °C. Read the absorbance (A) of the standard and samples at 550 (540-560) nm against Blank. Colour is stable for 30 minutes, protected from light.			

Note:

- Reaction volumes can be proportionally changed.
- For plasma or serum concentration > of 20 mg/dl (1190 µmol/l) dilute sample 1:2 with NaCl (9 g/l) solution and multiply the result by 2.
- For urine concentration > of 200 mg/dl (11.9 mmol/l) dilute sample 1:2 with distilled water (9 g/l) and multiply the result by 2.

CALCULATION OF RESULTS

Serum, plasma:

$$\text{Uric Acid, mg/dl} = \frac{\text{A sample}}{\text{A standard}} \times 6$$

Urine:

$$\text{Uric Acid, mg/24h} = \frac{\text{A sample}}{\text{A standard}} \times 600 \times \text{l/24h}$$

Conversion factor
Uric Acid [mg/dl] x 59.48 = Uric Acid [µmol/l]
Uric Acid [mg/dl] x 0.05948 = Uric Acid [mmol/l]

REFERENCE VALUES

Plasma/Serum		
	Female mg/dl (µmol/l)	**Male mg/dl (µmol/l)**
Adults	2.3-6.1 (137-363)	3.6-8.2 (214-488)
Children		
0-5 days	1.9-7.9 (113-470)	1.9-7.9 (113-470)
1-4 years	1.7-5.1 (101-303)	2.2-5.7 (131-470)
5-11 years	3.0-6.4 (178-381)	3.0-6.4 (178-381)
12-14 years	3.2-6.1 (190-381)	3.2-7.4 (190-440)
15-17 years	3.2-6.4 (190-381)	4.5-8.1 (190-440)
Urine		
≤ 800 mg/24h (4.76 mmol/l)	balanced diet	
≤ 600 mg/24h (5.57 mmol/l)	low purine diet	

Each laboratory should establish reference ranges for its own patients population.

ANALYTICAL PERFORMANCES

Precision

Within-run and between-run coefficients of variation have been calculated on replicates of three controls at different uric acid concentration. The obtained results are reported in the following table:

		Within-run		Between-run	
Sample	**Mean (mg/dl)**	**SD**	**%CV**	**SD**	**%CV**
Serum 1	4.6	0.08	1.8	0.17	3.8
Serum 2	12.1	0.22	1.8	0.53	4.4

Linearity

The assay is linear up to 20 mg/dl (1190 μmol/l).

Sensitivity

Test sensitivity, in terms of limit of detection, is 0.3 mg/dl (17.84 μmol/l).

Correlation

A correlation study comparing the present method with a commercial one gave the following results:

y = 1.1974x - 0.4471 mg/dl r = 0.9947

Interferences

Bilirubin	> 20 mg/dl
Hemoglobin	> 50 mg/dl
Triglycerides	> 2000 mg/dl
Ascorbic acid	> 30 mg/dl

PRECAUTIONS IN USE

The reagents contain inactive components such as preservatives (Sodium azide or others), surfactants etc. The total concentration of these components is lower than the limits reported by 67/548/EEC and 88/379/EEC directives about classification, packaging and labelling of dangerous substances. However, the reagents should be handled with caution, avoiding swallowing and contact with skin, eyes and mucous membranes. The use of laboratory reagents according to good laboratory practice is recommended.

Waste Management

Please refer to local legal requirements.

BIBLIOGRAPHY

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3. Chitto G, Fabi A, Franzini C, Galletta G, Leonardi A, Marelli M, Morelli AM: Variabilità biologica intra-individuo: rassegna della letteratura, contributo sperimentale e considerazioni critiche. Biochimica Clinica, 1994; 18, 10:673.
4. NCCLS Document, "Procedures for the collection of arterial blood specimens", Approved Standard, 3rd Ed. (1999).
5. EU-Dir 1999/11 Commission Directive of 8 March 1999 adapting to technical progress the principles of good laboratory practice as specified in Council Directive 87/18/EEC.