Uric acid

Reagent for quantitative In-vitro-determination of uric acid in serum / plasma

HSR 342

Order No. **HSR 342**
Content: 40 tests

Method [1,2]
Enzymatic colorimetric test, Uricase-PAP method

Sample material
Serum, haeparinised or EDTA plasma
Stability of uric acid in serum/plasma
at +2°C to +8°C: 5 days
at +15°C to +25°C: 3 days

Reagent
Contents / concentrations:
1. Starter reagent (caps in PE-bottle)
   Uricase > 500 U/L, Peroxidase (POD) > 750 U/L,
   4-Aminophenazone 0.23 mmol/L
2. Buffer solution (pre-portioned in round cuvettes)
   4-Chlorophenol 1.8 mmol/L, Sodium azide < 0.1 %;
   Triton X-100 < 1%, PIPES-buffer pH 7.8, 20 mmol/L,
   nonionic detergenz 7g/L, Natriumcholate 2,5 g/L

Safety information
The buffer solution (round cuvette) contains Sodium azide (<0.1 %) and Triton X-100. Do not swallow and avoid contact with skin and mucous membranes. If desired a safety data sheet will be provided.[3]

Storage and shelf life
Reagents can be kept at a temperature between +2°C and +8°C until the expiry date indicated on the packaging. Protect from direct sunlight and strong light.

Measurement conditions

Measurement devices:	Vario Photometer Dr. Lange Photometer
Meas. wavelength:	546nm
Temperature:	Room temperature

Measurement range
0.4 - 20 mg/dL (24 - 1190 µmol/L)
In case of exceeding these values, dilute the sample 1+1 with physiological saline solution. Multiply the result by 2.

Working instructions

Pipette into round cuvette:	
	Analysis
Serum / plasma	100 µL
Mix thoroughly. Leave it at room temperature for 10 minutes. Measure within 10 minutes.	

- Select the <**UA 342**> test.
- Set the photometer's zero point using a non-processed round cuvette (blank value).
- Remove cuvette after signal tone.
- Screw the cap from PE-bottle onto the cuvette with sample (analysis cuvette), dissolve the starter reagent by inverting several times.
- Leave it at room temperature for 10 minutes.
- Insert analysis cuvette, read the result.

Quality assurance
For quality assurance we recommend universal control sera from company Roche, www.roche.de:
PreciControl ClinChem Multi 1 / Multi 2 (4 x 5 mL)
Order-No.: 05 947 626 190 / 05 947 774 190
Ref.: Roche / Hitachi analyzers,
Method: plus (Uricase - PAP)

Reference values
Serum / plasma [4]

	mg/dL	µmol/L
Women	2.4 - 5.7	143 - 339
Men	3.4 - 7.0	202 - 416
24-h urine [7] : 0.25 - 0.75 g resp. 1.49 - 4.46 mmol		

Tips
□ The reagent solution, which needs to be shelved in the refrigerator, has to be warmed up to room temperature, before measurement.
□ In case of haemolytic or lipaemic sera it is recommendable to use a sample blank: For zero adjustment use the cuvette with sample material instead of the blank value cuvette. Afterwards start reaction with cap.

Summary
Uric acid is the final product of the purine metabolism. It arises due to the decomposition of endogenous and exogenous (with food ingested) purines in the organism. The excretion takes place at approx. 80 % via the kidney. If the serum-uric acid levels are too high, the released uric acid crystallises out and deposits in the tissue. [4,5]

Nowadays only enzymatic methods, which are based on the oxidation of uric acid via uricase, are used for the determination. The withdrawal of the uric acid concentration can be measured spectral-photometrically (UV test). As a matter of routine the PAP methods, which contain the Trinder reaction (H_2O_2, which was generated during the uricase reaction, converts into a dye), have proved of value. This measurement principle forms also the basis of the Diaglobal colorimetric test.

Measurement principle[4]

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

$$H_2O_2 + \text{4-Chlorophenol} + \text{4-Aminophenazone} \xrightarrow{\text{POD}} \text{Quinonimine dye}$$

The concentration of the quinonimine dye is propor- tional to the concentration of uric acid in serum / plasma and is measured photometrically at 546 nm.

Performance parameters
Specificity / interferences [6]
No interference due to bilirubin (< 20 mg/dL), lipaemia (triglycerides < 1000 mg/dL), and ascorbic acid in physiological concentrations (< 20 mg/l).
Haemoglobin falsifies too high values, in case of not considering the sample blank value.
Interferences due to pharmaceuticals: lowered values due to methyldopa (> 3 mg/l) and gentisine acid (> 15 mg/l) in concentrations above the therapeutic field.

Inaccuracy
The reproducibility was checked using human and control samples.

In series [n = 20]	Average [mg/dL]	Standard deviation [mg/dL]	VK [%]
Sample 1	4.29	0.10	2.3
Sample 2	9.77	0.16	1.6
From day to day [n = 20]	**Average [mg/dL]**	**Standard deviation [mg/dL]**	**VK [%]**
Sample 1	4.31	0.12	2.7
Sample 2	9.71	0.19	1.9

Analytic sensitiveness
Lower detection limit: 0.4 mg/dL (24 µmol/L)

Comparison of methods
A comparison of the Diaglobal test HSR 342 (y) and a commercially available test (x) based on the Uricase-PAP method resulted in the following correlation according to the Passing/Bablok[7] process:
y = 1.036x - 0.09
r = 0.992
n = 40
Concentration range: 1.6 - 18 mg/dL

Information on disposal
Waste code number 180106:
Vials with reagent are considered hazardous waste. Do not allow reagent to reach surface water or sewage system. Dispose of in accordance with official regulations.
Non-contaminated and completely empty packaging can be recycled.
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Bibliography
1. Henry RJ et al. Clinical Chemistry. 2nd rev. ed. New York: Harper & Row. 1974:541
2. Fossati P, Precipe L, Berti G. Clin Chem 1980;26:227
3. http://www.diaglobal.de/de/service/downloads/index.html
4. Thomas L. Labor und Diagnose. 4.Aufl. Marburg: Die Medizinische Verlagsgesellschaft 1995: 247
5. Rick W. Klinische Chemie und Mikroskopie. 6.Aufl. Berlin Heidelberg: Springer Verlag 1972: 250
6. Sonntag O. Arzneimittelinterferenzen. Stuttgart, New York: Thieme Verlag, 1985:43
7. Passing H, Bablok W. A new biometric procedure for testing the equality of measurements from two different analytical methods. J Clin Chem Clin Biochem. 1983; 21:709