SPINREACT

CE URIC ACID

Uric acid

Uricase -POD. Enzymatic colorimetric

Quantitative determination of uric acid
IVD

Store at 2-8ºC

PRINCIPLE OF THE METHOD

Uric acid is oxidized by uricase to allantoine and hydrogen peroxide ($2H_2O_2$), which under the influence of POD, 4–aminophenazone (4-AP) and 2-4 Dichlorophenol sulfonate (DCPS) forms a red quinoneimine compound:

$$\text{Uric acid} + 2H_2O + O_2 \xrightarrow{\text{Uricase}} \text{Allantoine} + CO_2 + 2H_2O_2$$

$$2H_2O_2 + \text{4-AP} + \text{DCPS} \xrightarrow{\text{POD}} \text{Quinoneimine} + 4H_2O$$

The intensity of the red color formed is proportional to the uric acid concentration in the sample[1,2].

CLINICAL SIGNIFICANCE

Uric acid and its salts are end products of the purine metabolism.
With progressive renal insufficiency, there is retention in blood of urea, creatinine and uric acid.
Elevate uric acid level may be indicative of renal insufficiency and is commonly associated with gout[3,4].
Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

REAGENTS

R 1 Buffer	Phosphate pH 7.4 2-4 Dichlorophenol sulfonate (DCPS)	50 mmol/L 4 mmol/L
R 2 Enzymes	Uricase Peroxidase (POD) Ascorbate oxidase 4 – Aminophenazone (4-AP)	60 U/L 660 U/L 300 U/L 1 mmol/L
URIC ACID CAL	Uric acid aqueous primary standard	6 mg/dL

PREPARATION

Working reagent (WR): Dissolve (→) the contents of one vial R 2 Enzymes in one bottle R 1 Buffer. Cap and mix gently to dissolve contents. (WR) is stable after reconstitution 1 month at 2-8ºC or 10 days at room temperature.

STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8ºC, protected from light and contaminations prevented during their use.
Do not use reagents over the expiration date.
Signs of reagent deterioration:
- Presence of particles and turbidity.
- Blank absorbance (A) at 520 nm ≥ 0,16.

ADDITIONAL EQUIPMENT

- Spectrophotometer or colorimeter measuring at 520 nm.
- Matched cuvettes 1,0 cm light path.
- General laboratory equipment.

SAMPLES

- Serum or plasma[1]: Stability 3-5 days at 2-8ºC or 6 months at -20ºC.
- Urine (24 h)[1]: Stability 4 days at 15-25ºC, pH >8. Dilute sample 1/50 in distilled water. Mix. Multiply results by 50 (dilution factor).
  If urine is cloudy: warm the specimen to 60ºC for 10 min to dissolve precipitated urates and uric acid. Do not refrigerate.

PROCEDURE

1. Assay conditions:
   Wavelength: ..... 520 nm (490-550)
   Cuvette: ..... 1 cm light path
   Temperature: ..... 37ºC / 15-25ºC
2. Adjust the instrument to zero with distilled water.
3. Pipette into a cuvette [Note 4]:

	Blank	Standard	Sample
WR (mL)	1,0	1,0	1,0
Standard [Note 0,6] (µL)	–	25	–
Sample (µL)	–	–	25

4. Mix and incubate for 5 min at 37ºC or 10 min at 15-25ºC.
5. Read the absorbance (A) of the samples and Standard, against the blank. The colour is stable for at least 30 minutes.

CALCULATIONS

Serum or plasma

$$\frac{\text{(A) Sample - (A) Blank}}{\text{(A) Standard - (A) Blank}} \times 6\ (\text{Standard conc.}) = \text{mg/dL uric acid in the sample}$$

Urine 24 h

$$\frac{\text{(A) Sample - (A) Blank}}{\text{(A) Standard - (A) Blank}} \times 6 \times \text{vol. (dL) urine 24 h} = \text{mg/24 h uric acid}$$

Conversion factor: mg/dL x 59,5= µmol/L.

QUALITY CONTROL

Control sera are recommended to monitor the performance of assay procedures: SPINTROL H Normal and Pathologic (Ref. 1002120 and 1002210).
If control values are found outside the defined range, check the instrument, reagents and calibrator for problems.
Each laboratory should establish its own Quality Control schema and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES[5]

Serum or plasma:

Women	2,5 - 6,8 mg/dL	≡	149 – 405 µmol/L
Men	3,6 - 7,7 mg/dL	≡	214 – 458 µmol/L
Urine:	250 - 750 mg/24 h	≡	1,49 - 4,5 mmol/24 h

These values are for orientation purpose; each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS

Measuring range: From *detection limit* of 0,00 mg/dL to *linearity limit* of 40 mg/dL.
If the results obtained were greater than linearity limit, dilute the sample 1/2 with NaCl 9 g/L and multiply the result by 2.
Precision:

	Intra-assay (n=20)		Inter-assay (n=20)	
Mean (mg/dL)	4,74	10,55	4,73	10,50
SD	0,02	0,03	0,13	0,29
CV (%)	0,50	0,30	2,67	2,77

Sensitivity: 1 mg/dL = 0,02930.
Accuracy: Results obtained using SPINREACT reagents (y) did not show systematic differences when compared with other commercial reagents (x).
The results obtained using 50 samples were the following:
Correlation coefficient (r): 0,97137.
Regression equation: y=1,162 x + 0,14186.
The results of the performance characteristics depend on the analyzer used.

INTERFERENCES

No interferences were observed to bilirubin up to 170 µmol/L, hemoglobin up to 130 mg/dL and ascorbic acid up to 570 µmol/L[6].
A list of drugs and other interfering substances with uric acid determination has been reported[3,4].

NOTES

1. URIC ACID CAL: Proceed carefully with this product because due its nature it can get contamined easily.
2. Calibration with the aqueous standard may cause a systematic error in automatic procedures. In these cases, it is recommended to use a serum Calibrator.
3. Use clean disposable pipette tips for its dispensation.
4. **SPINREACT has instruction sheets for several automatic analyzers. Instructions for many of them are available on request.**

BIBLIOGRAPHY

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4. Young DS. Effects of disease on Clinical Lab. Tests, 4th ed AACC 2001.
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PACKAGING

Ref. 1001010	Cont	R1: 10 x 20 mL, R2:10 → 20 mL, CAL: 1 x 5 mL
Ref. 1001011		R1: 10 x 50 mL, R2:10 → 50 mL, CAL: 1 x 5 mL
Ref. 1001012		R1: 4 x 125 mL, R2:4 → 125mL, CAL: 1 x 5 mL
Ref. 1001013		R1: 4 x 250 mL, R2:4 → 250mL, CAL: 1 x 5 mL

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