URIC ACID

URICASE/PEROXIDASE

Intended use:
Enzymatic in vitro test for the quantitative determination of uric acid in human serum and plasma.

Summary:
Uric acid is the final product of purine metabolism in the human organism. Uric acid measurements are used in the diagnosis and treatment of numerous renai and metabolic disorders, including renal failure, gout, leukaemia, psoriasis, starvation or other wasting conditions, and of patients receiving cytotoxic drugs. The oxidation of uric acid provides the basic for two approaches to the quantitative determination of this purine metabolite. One approach is the reduction of phosphotungstic acid in an alkaline solution to tungsten blue, which is measured photometrically. The method is, however, subject to interferences from drugs and reducing substances other than uric acid.
A second approach, described by Praetorius and Poulson, utilizes the enzyme uricase to oxidise uric acid; this method eliminates the interferences intrinsic to chemical oxidation. Uricase can be employed in methods that involve the UV measurement of the consumption of uric acid or in combination with other enzymes to provide a colorimetric method.
The assay described here is a slight modification of the colorimetric method. The modifications were described by Siedel. The peroxide reacts with phenole and amino-antipyrine in the presence of peroxidase to form a quinonimine dye. The intensity of the red color is proportional to the uric acid concentration and is determined photometrically.

Test principle:
Colorimetric endpoint assay

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

Uricase cleaves uric acid to form allantoin and hydrogen peroxide.

$$2H_2O_2 + 4H^+ + \text{phenole} + \text{4-aminoantipyrine} \xrightarrow{\text{POD}} \text{quinonimine dye} + 4H_2O$$

The increase in absorbance is measured.

Reagent concentration:

R1:

Phosphate buffer pH 8.0	50 mmol/l
Chlorophenole	7.5 mmol/l
Uricase	300 U/l
POD	> 1 KU/l
4-aminoantipyrine	3 mmol/l
Preservative	

Preparation and stability:
Gently swirl until completely dissolved. DO NOT SHAKE.
This reagent is stable (protected from light!):
28 days at + 2°C to + 8°C

Specimen:
Serum/plasma:
Collect serum using standard sampling tubes.
Heparin, or EDTA-plasma
Stability: 5 days at +2°C to +8°C
6 months at - 20°C

Dilute urine samples manually with distilled water or 0.9% NaCl - solution (e.g. 1 + 10). Multiply the result by the appropriate dilution factor (e.g. 11).
Centrifuge samples containing precipitate before performing the assay.

Limitations - interference:
Criterion: Recovery within ±10% of initial values
Serum/ Plasma Icterus: No significant interference up to an index I of 55 (approximate conjugated and unconjugated bilirubin concentration 55 mg/dl)
Hemolysis: No significant interference up to an index H of 25 (approximate hemoglobin concentration: 25 mg/dl).
Lipemia (Intralipid): No significant interference up to an index L of 300 (approximate triglycerides concentration: 600 mg/dl). There is poor correlation between turbidity and triglycerides concentration.
Elevated levels of ascorbic acid produces false low values.
Uricase reacts specifically with uric acid. Other purine derivatives can inhibit the uric acid reaction.

Analytical sensitivity (lower detection limit):
Detection limit: 0.2 mg/dl
The lower detection limit represents the lowest measurable uric acid concentration that can be distinguished from zero.

Testing procedure:
Applications for automated systems are available on request
Materials provided
- Working solutions as described above

Additional materials required
- Calibrators and controls as indicated below
- 0.9% NaCl – solution

Manual procedure	
Wavelength:	510 nm
Temperature:	+37°C
Cuvette:	1 cm light path
Zero adjustment:	Reagent blank
	Sample / Calibrator
Sample / Calibrator	25 µl
R1	1000 µl
Mix and incubate 5 minutes. Read the absorbance against blank within 30 minutes.	
Calculation: (A sample/ A Calibrator) x Calibrator conc. = Uric acid in mg/dl	

Measuring /reportable range:
0.11 – 25.0 mg/dl
Determine samples having higher concentrations via the rerun function. On instruments without rerun function, manually dilute the samples with 0.9% NaCl or distilled/deionized water (e.g. 1 + 1). Multiply the result by the appropriate dilution factor (e.g. factor 2).
Unit conversion:
mg/dl x 59.5 = µmol/l
mg/dl x 0.059 = mmol/l

Expected values:
Serum/plasma:
Male: 3.5 - 7.2 mg/dl (210 - 420 umol/l)
Female: 2.6 - 6.0 mg/dl (150 - 350 umol/l)
Urine (Reference range according to Krieg and Colombo)
Morning urine
250-750 mg/24-h = 1.5 - 4.5 umol
Urine (Reference range according to Tietz)
Average diet: 250 - 750 mg/24h
Low purine diet:
Male: < 480 mg/24h
Female: < 400 mg/24h
High purine diet: < 1000 mg/24h
Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference range.
For diagnostic purposes the uric acid results should always be assayed in conjunction with the patient's medical history, clinical examinations and other findings.

Imprecision:
Reproducibility was determined using controls within run (n = 20).
The following results were obtained:

Within run			
Sample	Mean (mg/dl)	SD (mg/dl)	CV (%)
Sample1	4.34	0.029	0.67
Sample2	5.92	0.056	0.95
Sample3	11.63	0.062	0.53
Between day			
Sample	Mean (mg/dl)	SD (mg/dl)	CV (%)
Sample1	4.73	0.097	2.05
Sample2	6.78	0.116	1.71
Sample3	11.03	0.315	2.86

Method comparison:
A comparison of the BIOANALYTIC UA (y) with a commercial obtainable assay (x) gave with 38 samples the following result (mg/dl):
y = 0.999 x – 0.034; r = 0.997

URIC ACID

URICASE/PEROXIDASE

Quality Control:
Control Serum:

BIOCON N	5 x 5 ml	#B10814
BIOCON P	5 x 5 ml	#B10817

The control intervals and limits must be adapted to the individual laboratory and country-specific requirements. Values obtained should fall within established limits. Each laboratory should establish corrective measures to be taken if values fall outside the limits.

Calibration:
S1: 0.9% NaCl

S2: BIOCAL H	5 x 3 ml	#B11895

Calibration frequency:
A two-point-calibration is recommended in case of:
1-change of lot
2- quality control requirements

Literature:
1. Colombo J-P (ed) Klinisch - chemische Urindiagnostik. Rotkreuz: Labolive-Verlagsgesellschaft 1994:180
2. DiGiorgio J; Henry RJ, et al eds. Clinical Chemistry: Principles and Technics. 2nd ed. New York NY: Harper and Row; 1974:532.
3. Elking SM, Kabat HF. Am Soc Hosp Pharm. 1969;25:485.
4. Glick MR. Ryder KW. Jackson SA. Graphical Comparisons of Interferences in Clinical Chemistry Instrumentation, Clin Chem 1986;32:470-474
5. Greiling H, Gressner AM, eds. Lehrbuch der Klinischen Chemie und Pathobiochemie, 3re ed. Stuttgart/New York: Schattauer Verlag; 1995.
6. Guder WG, Narayanan S, Wisser H, Zawta B, List of Analytes ; Pre analytical Variables. Brochure in Samples: From the Patient to the Laboratory. Darmstadt :GIT Verlag 1996
7. Haug HG. Diagnostik. 1972;18:137.
8. Kageyama N. A direct colorimetric determination of uric acid in serum and urine with uricase-catalase system. Clin Chim Acta 1971;31:421-426.
9. Kaiser E, et.al. Wiener Klin Wschr. 1972;84:217.
10. Keller H, ed. Klinisch-chemische Labordiagnostik für die Praxis, 2nd ed. Stuttgart/New York: Georg Thieme Verlag,1991.
11. Kim EK,wadel LD, sunderland MLE et al Observations on Diagnostic Kits for the Determination of Uric Acid. Clin Biochem. 1971;4:279-286.
12. Krieg M et al Vergleichende quantitative Analytik klinisch-chemischer Kenngrößen im 24 Stunden –Urin und Morgenurin. J Clin Chem Clin Biochem 1986;24:863-869.
13. Kueffer H. Therap Umschau.1971;28:669.
14. Praetorius E Poulsen H. Enzymatic Determination of Uric Acid with Detailed Directions. Scandinav J Clin Lab Investigation 1953;3:273-280.Mac Kay EM Mac Kay LL J Clin Invest 1927;4:295
15. Rice EW Gorgan BS. Clin chem. 1962;8:181.
16. Sing HP, et al Clin Chem. 1972;18:137.
17. Thefeld W, Hoffmeister H, Busch EW et al. Normalwerte der Serumharnsäure
in Abhängigkeit von Alter und Geschlecht mit einem neue enzymatischen Harnsäurefarbtest. Dtsch Med Wschr 1973;98:380-384.
18. Tietz NW. Clinical Guide to Laboratory Tests 3rd ed. Philadelphia, Pa. WB Saunders Company. 1995:624-629.
19. Town MH, Gehm S, Hammer B, Ziegenhorn J. J Clin Chem Clin Biochem 1985; 23:591.
20. Young DS, et al Clin Chem 1972;18:1042

Order information (Cat No.) :

CC495	BUAC500	B25295	B30295	B34295	B80297
OL495	BUAC250	B25296	B30296	B35295	
AB495	B21295	B27295	B31295	B36295	
KL495	B21296	B27296	B32295	B37295	
SH495	B22295	B28295	B33295	B80295	
CR495	B24295	B28296	B33296	B80296	

SYMBOLS

IVD	for in vitro diagnostic use only
LOT	lot of manufacturing
REF	code number
	storage at temperature interval
	expiration date (year/month)
	warning, read enclosed documents
	Read the directions

Manufacturer
Diaclinica Diagnostik Kimya.San.Tic.Ltd.Şti
Adress : İkitelli O.S.B Mutsan San.Sit. M4 Blok No:17-19 Başakşehir/İSTANBUL
Tel:+90(212) 549 33 88- Fax:+90 (212) 549 55 50
Web :www.diaclinica.com

Rev:V7.0104 / Date : 01.17