Uric Acid

Enzymatic. Colorimetric
URICASE-POD
Liquid

Store at 2-8°C

CE

IVD

Configuration

REF	HBL020	HBL020A	HBL020M
VOL	2x125 ml	8x125 ml	8x30 ml
Reagent 1	1x125 ml	4x125 ml	4x30 ml
Reagent 2	1x125 ml	4x125 ml	4x30 ml
Standard	1x5 ml	4x5 ml	-
Instrument	Universal	Universal	Mindray BS-120, BS-200, BS-200E, BS-230, BS-240, BS-240 Pro

Intended use

Quantitative determination of uric acid in human serum, plasma or urine.
For *in vitro* diagnostic use only.
For professional use only.

Clinical significance

Uric acid is the end-product of purine metabolism. Nearly half of the total uric acid is eliminated and replaced each day by way of urinary excretion and through microbial degradation in the intestinal tract. Increased uric acid levels are commonly associated with both nitrogen retention and urea, creatinine, and other non-protein constituents. The quantification of uric acid is an aid in the diagnosis of gout, decreased renal function, myeloproliferative disorders, and other conditions in which the cause for the hyperuricemia is not well known[1,5,6].
Clinical diagnosis should not be made on a single test result; it should integrate clinical and laboratory data.

Principle

Uric acid is oxidized by uricase to allantoin and hydrogen peroxide, which under the influence of POD, oxidizes 2-4-dichlorophenol sulfonate (DCPS) and 4-Aminophenazone (4-AP) to form a red quinoneimine compound.

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

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

The quantity of this red quinoneimine formed is proportional to the uric acid concentration in the sample[1,2].

Reagent composition

Reagent 1 Buffer	Pipes pH 7,0 ..... 50 mmol/l 4-AP ..... 1 mmol/l Ascorbate oxidase (ASO) ..... 1000 U/l Peroxidase (POD) ..... 3800 U/l
Reagent 2 Enzymes	Pipes pH 7,0 ..... 50 mmol/l DCPS ..... 2,5 mmol/l Uricase (UAO) ..... 250 U/l
Standard	Uric Acid aqueous ..... 6 mg/dl

Preparation

Mix equal volumes of R1 Buffer and R2 Enzymes. This working reagent is stable for 2 months at 2-8°C or 2 weeks at room temperature (15-25°C).

Storage and stability

All the components of the kit are stable at 2-8°C up to the date of expiration as specified, when stored tightly closed, protected from light and contaminations prevented during their use. Handle standard very carefully to prevent contamination.
The reagents should be clear solutions. If turbidity or precipitation has occurred or if blank absorbance of the working reagent at 510 nm ≥ 0,12, the reagents should be discarded.

Additional material required but not provided

- Spectrophotometer or colorimeter measuring at 510 nm
- Matched cuvettes 1,0 cm light path
- General laboratory equipment

Samples

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

Procedure

1. Wavelength 510 nm (490-550); Temperature 37°C/15-25°C; Cuvette 1 cm light path.
2. Adjust the instrument to zero with distilled water
3. Pipette into a cuvette:

	Blank	Standard	Sample
Standard[Note]	---	25 µl	---
Sample	---	---	25 µl
Working reagent	1,00 ml	1,00 ml	1,00 ml

Mix, incubate for 10 min at room temperature or 5 min at 37°C. Read the absorbance (Abs) of sample and of standard against blank. The color is stable for at least 45 minutes.

Calculation

Serum or plasma: Uric acid (mg/dl):

$$\text{Uric acid (mg/dl)} = \frac{Abs_{Sample} - Abs_{Blank}}{Abs_{Standard} - Abs_{Blank}} \times 6\ (\text{stand. conc.})$$

Urine 24h: Uric acid (mg/24h):

$$\text{Uric acid (mg/24h)} = \frac{Abs_{Sample} - Abs_{Blank}}{Abs_{Standard} - Abs_{Blank}} \times 6\ (\text{stand. conc.}) \times \text{Vol (dl) urine 24h}$$

Conversion factor: mg/dl x 59,48 = µmol/l.

Quality control

Control sera are recommended to monitor the performance of assay procedures. If control values are found outside the defined range, check the instrument, reagents and calibrator for problems. Each laboratory should establish its own QC scheme and corrective actions if controls do not meet the acceptable tolerances.
Normal and pathological human (HBC01, HBC02) sera are available.

Reference values

Serum or plasma:

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

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

Performance characteristics

Measuring range: from 0,15 mg/dl (detection limit) to 25 mg/dl (linearity limit). If the obtained results are greater than 25 mg/dl, dilute the sample 1:2 with saline solution, repeat the determination, and multiply the result by factor 2.
Precision:

	Intra-assay (n=20)		Inter-assay (n=20)	
Mean (mg/l)	4,37	10,00	4,38	10,10
SD	0,06	0,16	0,04	0,10
CV (%)	1,42	1,65	0,98	1,00

Sensitivity: 1 mg/dl = 0,0355 Abs
Accuracy: Results obtained using CYPRESS DIAGNOSTICS reagents did not show systematic differences when compared with other commercial reagents.
The results of the performance characteristics depend on the analyzer used.

Interferences

No interferences were observed to bilirubin up to 10 mg/dl, hemoglobin up to 130 mg/dl and ascorbic acid up to 10 mg/dl[2]. A list of drugs and other interfering substances with uric acid determination has been reported by Young et al[3,4].

Notes

1. Calibration with the aqueous standard may cause a systematic error in automatic procedures. In this case, it is recommended to use a serum calibrator (HBC03).
2. For best use of this kit on a Cypress Diagnostics analyzer (CYANSmart, CYANStart, CYANExpert 130) or Mindray analyzer (Mindray BS-120, BS-200, BS-200E) we kindly advise to follow the application sheets of the respective analyzer. Please log in to our website (www.diagnostics.be) as a registered user to download the latest application sheets, which are located under the section of the corresponding analyzer.

Bibliography

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