

URIC ACID

Diagnostic reagent for determination of Uric Acid concentration.

Liquid. Monoreagent. Store at +2/+8°C. For in Vitro Diagnostic Use. Do not freeze

| Ref No | Pack |
|--------|-------|
| MH-302 | 60 mL |

Changes made in the instructions for use are marked as grey.

INTENDED USE

The test is applied for the quantitative determination of uric acid in serum or plasma.

GENERAL INFORMATION

In humans, uric acid (2,6,8-trihydroxy purine) is the main product of the catabolism of the purine nucleosides adenosine and guanosine. Purines resulting from the catabolism of dietary nucleic acids are directly converted to uric acid. However, a larger proportion of the purines excreted as uric acid originate from the breakdown of endogenous nucleic acids; dietary sources contribute additionally 300 mg while the daily synthesis rate of uric acid is approximately 400 mg. In men consuming a purine-free diet, the total body pool of exchangeable uric acid is about 1200 mg, while in women it is about 600 mg. On the contrary, the uric acid pool can increase to levels of 18,000 to 30,000 mg in patients with gouty arthritis and uric acid accumulation in tissue. About 75% of uric acid is excreted through the urine; most of the rest is secreted into the gastrointestinal tract, where it is broken down by bacterial enzymes into allantoin and other compounds. The physicochemical properties of uric acid are important in the assessment of uric acid concentrations in the circulation, tissues and kidneys. The initial pKa of uric acid is 5.57; above this pH uric acid exists mainly as urate ion, which is more soluble than uric acid. At urine pH below 5.75, uric acid is the predominant form. Considering the circulating ionization form, it is recommended that urate is preferred rather than uric acid when giving test results those in serum ¹

Hyperuricemia can result from increased synthesis or decreased excretion. Increased synthesis is secondary to increased purine load, mostly due to increased dietary purine intake or increased nucleic acid turnover.

Increased nucleic acid turnover is a feature of hematologic malignancies and massive tissue destruction following trauma, hypoxia or radiotherapy and chemotherapy.

Increased uric acid synthesis also occurs with excessive alcohol consumption. Increased uric acid synthesis may also be due to a primary disorder (idiopathic or inborn errors of metabolism such as Lesch-Nyhan syndrome). Decreased excretion may be idiopathic or secondary to kidney disease. Renal excretion of uric acid will be reduced if there is a decrease in glomerular filtration, inhibition of tubular secretion or increased tubular reabsorption. Reasons for decreased excretion include

renal failure, lead poisoning and the use of thiazide diuretics. At physiologic pH, uric acid exists mainly as the urate ion. Monosodium urate, sparingly soluble in extracellular fluids, is the predominant form. Any increase in concentration leads to the risk of supersaturated solutions and crystal formation. Thus, the deposition of urate crystals in the synovial space and soft tissue around the joint leads to gout. The crystals cause an inflammatory reaction. Acute gout attacks can occur after sudden decreases or sudden increases in plasma uric acid concentration. A sudden increase in urate production can lead to diffuse crystal formation in the renal tubules and acute obstructive nephropathy. At pH <5.75, the predominant form is uric acid, which has even poorer solubility. Chronic hyperuricosuria is associated with the formation of uric acid kidney stones. The risk is increased in people who consistently have acidic urine without an alkaline pH after a meal. Hyperuricosuria is treated by reducing the purine content of the diet and with drugs such as allopurinol, which reduces uric acid synthesis by inhibiting xanthine oxidase, or sulfapyrazone, which increases uric acid excretion by inhibiting tubular reabsorption.²

In pre-eclampsia, plasma uric acid concentrations are increased to a greater extent than in normal pregnancy, probably due to decreased renal perfusion, increased uric acid load due to placental fragmentation, and increased tubular reabsorption. Concentrations correlate with severity of disease, and higher levels are associated with poor fetal outcomes.^{3,4}

However, studies have shown that the sensitivity and specificity of uric acid measurements in diagnosing pre-eclampsia in individual patients is poor.⁵

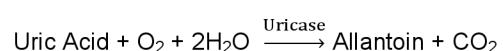
Hypouricemia can occur in severe liver disease, with azathioprine therapy, following over-treatment with allopurinol or uricosuric drugs, or due to tubular damage that reduces reabsorption (Fanconi syndrome, radiocontrast media and other toxins). Inherited defects in xanthine oxidase or the combined xanthine oxidase/sulfite oxidase complex are also associated with low uric acid concentrations.²

TEST PRINCIPLE

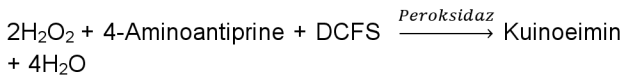
Enzymatic colorimetric method

The test principle is based on a two-step reaction:

First step: The reaction catalyzed by the enzyme uricase cleaves uric acid into hydrogen peroxide with allantoin.



In the second step: hydrogen peroxide formed in the first step reacts with dichlorophenolsulfonate (DCFS) and 4-aminoantipyrine in a reaction catalyzed by the enzyme peroxidase (POD) to form a quinonimine dye. The intensity of the color formed is determined photometrically by the absorbance value measured at a wavelength of 520 nm and is proportional to the uric acid concentration.



REAGENT COMPONENTS

| | |
|-------------------------|----------------|
| Phosphate | : ≤ 120 mmol/L |
| Detergent | : ≤ 1.8 g/L |
| Dichlorophenolsulfonate | : ≤ 4.4 mmol/L |
| Uricase | : > 0.12 U/mL |
| Ascorbate oxidase | : > 5 U/mL |
| Peroxidase | : > 1 U/mL |
| 4-aminoantipyrine | : ≤ 0.6 mmol/L |
| pH | : 7.8 |

Annotation:

- The dichlorophenolsulfonate in the reagent is a phenolic compound and produces products with higher molar absorptivity.⁶ This increases the precision of the assay and also allows smaller sample volumes to be used, thus reducing interference from endogenous phenolic compounds thought to occur in the plasma of patients with renal impairment.⁷
- Ascorbate oxidase (L-ascorbate: oxygen oxidoreductase; EC 1.10.3.3) is commonly included to eliminate interference from the reducing agent ascorbate.²

REAGENT PREPARATION

Reagent is ready for use.

REAGENT STABILITY AND STORAGE

Reagents are stable at +2/+8°C till the expiration date stated on the label which is only for closed vials.

Once opened vials are stable for 30 days at +2/+8°C in optimum conditions. On board stability is strongly related to auto analyzers' cooling specification and carry-over values.

Reagent stability and storage data have been verified by using Clinical and Laboratory Standards Institute (CLSI) EP25-A protocol.⁸

SAMPLE REQUIREMENTS

Serum, heparinised or EDTA plasma and urine can be used and are collected according to the standard procedures. Oxalate citrate and fluoride may cause decrease in uric acid. Multiple sample freezing and thawing should be avoided. The sample should be homogenized before testing.

Annotation:

- Plasma samples have been reported to have slightly higher (0.01 mmol/L) uric acid concentrations compared to serum.⁹
- EDTA may cause significant interference in some methods and may require the use of different reference ranges.¹⁰
- Some publications reported that the separating gel in tubes had no significant effect on uric acid measurement, but did not specify the analytical methods used.^{11,12}

Uric Acid activity stability in serum and plasma²⁶:

7 days at +2/+8°C
3 days at +20/+25°C
6 month at -20°C

Urine²⁶ (upon NaOH addition):

4 days at +20/+25°C

Annotation:

- Uric acid is stable in urine for 3 days at room temperature in the absence of bacterial contamination. Samples should not be refrigerated and acidified collections are not suitable as urate precipitation will occur. The pH of urine samples can be adjusted to > 8.0 by adding sodium hydroxide to prevent precipitation.¹⁰

CALIBRATION AND QUALITY CONTROL

Calibration: The assay requires the use of an Arcal Auto Calibrator. We recommend:

Arcal Auto Calibrator

Ref.No: VT-003

Calibration stability depends on the application characteristics and cooling capacity of the autoanalyzer used. Calibration stability is 30 days.

Control: Commercially available control material with established values determined by this method can be used. We recommend:

ARCON N (Level I Control) Lyophilized

Ref.No: VT-001

ARCON P (Level II Control) Lyophilized

Ref.No: VT-002

At least two level controls must be run once in every 24 hours. Each laboratory should determine its own quality control scheme and procedures. If quality control results are not within acceptable limits, calibration is required.

REFERENCE INTERVALS / MEDICAL DECISION LEVELS

Plasma uric acid results may be reported in mmol/L or $\mu\text{mol/L}$. Urine uric acid results are usually reported in mmol/L.

Serum / plasma²⁷

| Age | mg/dL | mmol/L |
|-----------------------|-----------|------------|
| 0 - 14 days | 2.8- 12.7 | 0.17- 0.75 |
| 15 days - < 1 year | 1.6- 6.3 | 0.09- 0.37 |
| 1 - < 3 years | 1.8- 4.9 | 0.11- 0.29 |
| 3 - < 5 years | 2.0- 4.9 | 0.12- 0.29 |
| 5 - 8 years | 1.9- 5.0 | 0.11- 0.30 |
| 9 - 10 years | 2.4- 5.5 | 0.14- 0.32 |
| 11 - 12 years | 2.6- 5.8 | 0.15- 0.34 |
| 13 - 79 years (men) | 3.7- 7.7 | 0.22- 0.45 |
| 13 - 79 years (women) | 2.5- 6.2 | 0.15- 0.37 |

24-hour urine²⁸ : 250 - 750 mg/24 hour

Conversion factors: Uric Acid, mg/dL \times 0.059 = Uric Acid, mmol/L

Annotation:

- Plasma uric acid concentrations gradually increase with age; concentrations in 60-year-olds are approximately 10% higher than in 20-year-olds.
- Plasma uric acid concentrations are lower in women than in men, but after menopause concentrations may increase to levels similar to those in men. During pregnancy, uric acid concentrations fall during the first trimester, but then increase from 24 weeks onwards and may reach higher levels than in non-pregnant women.³
- A slight diurnal variation has been reported (lowest at 11:00, highest at 08:00 and 17:00). While some studies have reported significant decreases after meals, not all studies support this information.⁹
- Urinary uric acid excretion is associated with dietary intake of purines, with a 20% to 25% reduction in a purine-free diet.³

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary, determine its own reference range.

Reference interval has been verified by using CLSI EP28-A3c protocol.¹³

PERFORMANCE CHARACTERISTICS

Measuring Interval

According to CLSI EP34-ED1:2018, "Measuring Interval" refers to the interval where the analyte concentration is measured with intended accuracy in terms of medical and laboratory requirements without dilution, concentrating or any kind of pre-treatment that is between the analyte's

lower limit of quantitation (LLoQ) and upper limit of quantitation (ULoQ).¹⁴

The determined analytic measuring interval for Uric Acid is 1 – 25 mg/dL.

Detection Capability

Limit of Detection (LoD): 0.6 mg/dL

Limit of Quantitation (LoQ): 1 mg/dL

Note: LoQ values are based on Coefficient of Variation Percentage (CV) \leq 20%.

LoD and LoQ values have been verified by using CLSI EP17-A2:2012 protocol.¹⁵

Linearity

This method shows measurement linearity in the activities up to 25 mg/dL.

Autoanalyzer's auto-dilution system can be used if the concentrations have higher values. See device manual for further information.

For manual dilution procedure, dilute the sample 10-fold using 0.90% isotonic. After the dilution, multiply the result of rerun sample by the dilution factor. Do not report the sample result after dilution if it is marked as lower than the linear lower limit. Rerun with a suitable dilution.

Linearity Studies data have been verified by using CLSI EP06-A:2003 protocol.¹⁶

Precision

Running system has been developed according to 20x2x2 "The Single Site" protocol. Repeatability and Within-Laboratory Precision/Within-Device values have been obtained according to the running results.

According to the protocol in use, 2 separate runs per day have been made for 20 days (no obligation for being consecutive days). This protocol has been applied to each low and high samples separately and 80 results have been obtained for each one. Statistically, the results have been obtained using 2-factor Nested-ANOVA model.¹⁷

Repeatability (Within Run) and Repeatability (Day to Day) SD (standard deviation) and CV% values of Uric Acid have been given in the table 1 and 2 respectively.

Table 1. Uric Acid Repeatability (Within Run) results obtained from samples in two different concentrations

| Mean Concentration | SD | CV% | n |
|--------------------|------|------|----|
| 5.18 mg/dL | 0.06 | 1.15 | 80 |
| 8.00 mg/dL | 0.04 | 0.50 | 80 |

Note: This working system has been named "Within-Run Precision" in the previous CLSI - EP05-A2 manual.¹⁸

Table 2. Uric Acid Repeatability (Day to Day) results obtained from samples in two different concentrations

| Mean Concentration | SD | CV% | n |
|--------------------|------|------|----|
| 5.18 mg/dL | 0.09 | 1.73 | 80 |
| 8.00 mg/dL | 0.19 | 2.37 | 80 |

Note: This working system has been named "Total Precision" in the previous CLSI - EP05-A2 manual.¹⁸

Method Comparison

As a result of the statistical evaluation of the method comparison data:

Passing-Bablok equation:¹⁹

$$y = 0.97x + 0.04 \text{ mg/dL}$$

$$r = 0.99$$

Interference

Endogenous interferant and analyte concentrations that have been used in the Uric Acid scanning tests has been determined according to "CLSI EP37-ED1:2018" and "CLSI EP07-ED3:2018" manuals.^{20,21}

The total acceptable error rate, which is going to be used to detect whether the observed differential value obtained from Uric Acid interference scanning test is appropriate, is determined as $\pm 25\%$.²²

In Uric Acid test results, no significant interaction has been observed in the determined endogenous interferant and analyte concentrations or between interferants and analyte.

| Interferant and Concentration | Uric Acid Target (mg/dL) | N* | %Observed Recovery |
|-------------------------------|--------------------------|----|--------------------|
| Bilirubin 7,12 mg/dL | 4,78 | 3 | 90 |
| Lipemia 1336 mg/dL | 4,44 | 3 | 102 |

* Total acceptable error rate determined as interference limit and repeatability (within run) pre-detected for the related method were used for the calculations of how many times the control and test samples prepared as a serum pool are going to be run repetitively. In the calculations, the accepted error rate for type 1 (α error) was 5% and for type 2 (β error) was 10% (90% power).²¹

Annotation:

- Xanthine, the direct precursor of uric acid, is a competitive inhibitor of uricase. Endogenous xanthine concentrations are rarely high enough to interfere with uric acid measurement methods. However, interference has been reported in a patient with increased xanthine concentrations following allopurinol treatment during

chemotherapy.²³ However, xanthine interference can be prevented by increasing the uricase concentration in end point measurement methods.⁶

- Copper, mercury, cyanide and formaldehyde can interfere with uricase but are not expected to be elevated to significant concentrations except in contaminated urine samples.⁶
- Rasburicase is a recombinant uricase used to prevent acute renal failure due to hyperuricemia in patients with high tissue turnover, such as hematological malignancy or tumor lysis syndrome following chemotherapy. The elimination half-life of the enzyme is 19 hours. This drug continues to oxidize uric acid in plasma in vitro. The drug manufacturer recommends that samples be collected in pre-chilled heparinized tubes, transported on ice, centrifuged at 4°C and analyzed within 4 hours to minimize uric acid loss.^{24,25}
- Non-hemolyzed and non-lipemic samples should be used.

It should be noted that endogenous interferants, as well as various medicines and metabolites, anticoagulants (e.g. Heparin, EDTA, citrate, oxalate) and preservatives (e.g. sodium fluoride, iodoacetate, hydrochloride acids) such as additives, materials that may contact with samples during collection and processing (serum separator devices, sample collection containers and contents, catheters, catheter wash solutions, skin disinfectants, hand cleaners and lotions, glass washing detergents, powder gloves), dietary substances known to affect some specific tests (caffeine, beta-carotene, poppy seeds, etc.), or some substances present in a sample that cause foreign proteins (heterophilic antibodies, etc.), autoimmune response (autoantibodies, etc.), or due to malignancy (for example, interference by paraproteins with phosphate testing and indirect ion selective electrode methods) may show some negative effects that will cause various attempts and some misjudgements.²¹

These performance characteristics have been obtained using an autoanalyzer. Results may vary slightly when using different equipment or manual procedures.

WARNINGS AND PRECAUTIONS

IVD: For in Vitro Diagnostic use only.

Do not use expired reagents.

Reagents with two different lot numbers should not be interchanged.

For professional use.

Follow Good Laboratory Practice (GLP) guidelines.

CAUTION: Human source samples are processed with this product. All human source samples must be treated as potentially infectious materials and must be handled in accordance with OSHA (Occupational Safety and Health Administration) standards.

Danger

EUH032 :Releases a very toxic gas if contacts with acid.

H317 :May cause allergic skin reaction.

Precaution

P280 :Use protective gloves / clothes / glasses / mask.

P264 :Wash your hands properly after using.

P272 :Contaminated work clothes should not be allowed to be used outside of the workplace.

Intervention

P302+P352 :Wash with plenty of water and soap if it contacts with skin.

P333+P313 :Seek medical help if it irritates your skin or develops rash.

P362+P364 :Remove contaminated clothes and wash properly before using.

Disposal

P501 :Dispose the vials and contents according to the local regulations.

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SYMBOLS

IVD

In Vitro Diagnostic Medical Device

LOT

Lot Number

R1

Reagent 1

GTIN

Global Trade Item Number

REF

Reference Number

GLP

Good Laboratory Practice

FOR USE WITH

Identifies Products to Be Used Together

PRODUCT OF TURKEY

Product of Turkey



Manufacturer



Expiration Date



Temperature Limits



Consult Instructions for Use



Caution



Number of Tests