04859332001V14.0

UA2

cobas®

Uric Acid ver.2

Order information

REF	CONTENT	Analyzer(s) on which kit(s) can be used
04657608190	Uric Acid ver.2 (4 × 100 tests)	**cobas c** 111

Materials required (but not provided):

10759350190	Calibrator f.a.s. (12 × 3 mL)	Code 401	
12149435122	Precinorm U plus (10 × 3 mL)	Code 300	
12149443122	Precipath U plus (10 × 3 mL)	Code 301	
05117003190	PreciControl ClinChem Multi 1 (20 × 5 mL)	Code 391	
05947626190	PreciControl ClinChem Multi 1 (4 × 5 mL)	Code 391	
05117216190	PreciControl ClinChem Multi 2 (20 × 5 mL)	Code 392	
05947774190	PreciControl ClinChem Multi 2 (4 × 5 mL)	Code 392	

English

System information

UA2: ACN 700

UAU2: ACN 702

Intended use

In vitro test for the quantitative determination of uric acid in serum, plasma and urine on the **cobas c** 111 system.

Summary

Uric acid measurements, performed with this assay, in human serum, plasma and urine are used as aid in diagnosis and treatment of numerous renal and metabolic disorders associated with hyper- or hypo-uricemia.

Uric acid is the major final product of purine metabolism in the human organism. Purines from dietary nucleic acids are converted in the liver and small intestine to uric acid.[1] Uric acid is present as a normal intracellular component and in biological fluids. Chemically, it is a reducing agent and accounts for nearly half of the antioxidant activity in blood. Uric acid production is balanced between purine ingestion, de novo synthesis, reabsorption, and degradation. Two-thirds of uric acid is excreted renally, while one-third is eliminated through the gastrointestinal system. Serum uric acid levels increase physiologically and gradually over the course of human life and are strongly influenced by the diet.[1,2]

High serum levels of uric acid can adversely affect organ systems. Overproduction of uric acid, insufficient excretion of uric acid, or often a combination of both can lead to hyperuricemia.[3] Primary causes of hyperuricemia include idiopathic and hereditary metabolic disorders. Secondary causes of increased uric acid formation include excessive dietary intake of purines and increased nucleic acid turnover (e.g. in myeloproliferative disorders, lymphoproliferative disorders, psoriasis, sarcoidosis, hemolytic anemia, cytotoxic drug treatments). Major causes of decreased uric acid excretion are: acute or chronic kidney disease, increased renal tubular reabsorption, reduced tubular secretion, lead poisoning, preeclampsia, low doses of salicylate, thiazide diuretics, Down syndrome.[1]

Hyperuricemia is mostly asymptomatic, but persistent hyperuricemia and uric acid precipitation may lead to the accumulation of urate crystals in many tissues, resulting in either acute painful conditions, such as gout/tophaceous gout/gouty arthritis, urolithiasis, or, in severe cases, in uric acid kidney diseases.[4]

Hypouricemia is much less common than hyperuricemia. Hypouricemia is often defined as serum uric acid levels ≤ 2.0 mg/dL (0.12 mmol/L). It may be secondary to any one of a number of underlying conditions, such as severe hepatocellular disease with reduced purine synthesis or xanthine oxidase activity, defective renal tubular reabsorption of uric acid (congenital or acquired), overtreatment of hyperuricemia, treatment with uricosuric drugs and cancer chemotherapy with 6-mercaptopurine or azathioprine.[1,5]

Phosphotungstic acid (PTA), uricase, and HPLC-based methods have been described for measuring uric acid. PTA methods are now rarely used.[1,6] The uricase-based method utilizes the enzyme uricase to oxidize uric acid.[7] 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 assay.[1]

The colorimetric method developed by Town, et al. involves initial sample incubation with a reagent mixture containing ascorbate oxidase and a clearing system. In this test system it is important that any ascorbic acid present in the sample is eliminated in the preliminary reaction; this precludes any ascorbic acid interference with the subsequent peroxidase (POD) indicator reaction. Upon addition of the starter reagent, oxidation of uric acid by uricase begins.[8]

The Roche assay described here is a slight modification of the colorimetric method described above. In this reaction, the peroxide reacts in the presence of peroxidase (POD), N-ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methylaniline (TOOS), and 4-aminophenazone to form a quinone-diimine dye. The intensity of the red color formed is proportional to the uric acid concentration and is determined photometrically.

Test principle

Enzymatic colorimetric test

Uricase cleaves uric acid to form allantoin and hydrogen peroxide.

$$\text{Uric acid} + 2\,H_2O + O_2 \xrightarrow{\textit{uricase}} \text{allantoin} + CO_2 + H_2O_2$$

$$2\,H_2O_2 + H^+ + \text{TOOS}^{a)} + \text{4-aminophenazone} \xrightarrow{\textit{peroxidase}} \text{quinone-diimine dye} + 4\,H_2O$$

a) N-ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methylaniline

The color intensity of the quinone-diimine formed is directly proportional to the uric acid concentration and is determined by measuring the increase in absorbance.

Reagents - working solutions

R1 Phosphate buffer: 0.05 mol/L, pH 7.8; TOOS: 7 mmol/L; fatty alcohol polyglycol ether: 4.8 %; ascorbate oxidase (EC 1.10.3.3; zucchini): ≥ 83.5 µkat/L (25 °C); stabilizers; preservative

SR Phosphate buffer: 0.1 mol/L, pH 7.8; K-hexacyanoferrate (II): 0.3 mmol/L; 4-aminophenazone: ≥ 3 mmol/L; uricase (EC 1.7.3.3; Arthrobacter protophormiae): ≥ 83.4 µkat/L (25 °C); peroxidase (POD) (EC 1.11.1.7; horseradish): ≥ 50.0 µkat/L (25 °C); stabilizers; preservative

Precautions and warnings

For in vitro diagnostic use for health care professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:
Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:
Apply all relevant local disposal regulations to determine the safe disposal.

Safety data sheet available for professional user on request.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

UA2

Uric Acid ver.2

cobas®

Warning

H319 Causes serious eye irritation.

Prevention:

P264 Wash skin thoroughly after handling.

P280 Wear eye protection/ face protection.

Response:

P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P337 + P313 If eye irritation persists: Get medical advice/attention.

Product safety labeling follows EU GHS guidance.

Contact phone: all countries: +49-621-7590

Reagent handling

Ready for use

Inaccurate pipetting of reagent, leading to potentially erroneous results, may be caused by excessive foaming of reagent. Ensure that foam is removed from the surface of the reagent prior to setting the reagent in the analyzer.

Storage and stability

Shelf life at 2-8 °C:	See expiration date on reagent
On-board in use and refrigerated on the analyzer:	4 weeks

Specimen collection and preparation

For specimen collection and preparation only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable.
Serum
Plasma: Li-heparin, K_3-EDTA plasma

EDTA plasma values are approximately 7 % lower than serum values.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Urine
Assay urinary uric acid as soon as possible. Do not refrigerate. To prevent ureate precipitation in urine samples, add sodium hydroxide to keep the urine alkaline (pH > 8.0). To achieve stated uric acid stability, add NaOH prior to sample collection.
Urine samples are automatically prediluted 1:11 (1+10) with water by the instrument.

Centrifuge samples containing precipitates before performing the assay.

See the limitations and interferences section for details about possible sample interferences.

Stability in serum/plasma:[9]	7 days at 4-8 °C
	3 days at 20-25 °C
	6 months at -20 °C (± 5 °C)
Freeze only once.	
Stability in urine[9] (upon NaOH addition):	4 days at 20-25 °C

Materials provided

See “Reagents – working solutions” section for reagents.

Materials required (but not provided)

See “Order information” section

General laboratory equipment

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator’s manual for analyzer-specific assay instructions.

The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for serum, plasma and urine

cobas c 111 **test definition**

Measuring mode	Absorbance
Abs. calculation mode	Endpoint
Reaction direction	Increase
Wavelength A/B	552/659 nm
Calc. first/last	16/20
Unit	µmol/L
Serum/plasma	
Reaction mode	R1-S-SR
Urine	
Reaction mode	D-R1-S-SR
Predilution factor	11

Pipetting parameters

Serum/plasma/urine		Diluent (H_2O)
R1	72 µL	
Sample	3 µL	45 µL
SR	14 µL	
Total volume	134 µL	

Calibration

Calibrator	Calibrator f.a.s.
	Deionized water is used automatically by the instrument as the zero calibrator
Calibration mode	Linear regression
Calibration interval	Each lot and as required following quality control procedures

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Traceability: This method has been standardized against ID/MS.[10]

Quality control

Serum/plasma

For quality control, use control materials as listed in the “Order information” section. In addition, other suitable control material can be used.

Urine

Quantitative urine controls are recommended for routine quality control.

The control intervals and limits should be adapted to each laboratory’s individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Follow the applicable government regulations and local guidelines for quality control.

Calculation

The **cobas c** 111 analyzer automatically calculates the analyte concentration of each sample.

Conversion factors: µmol/L × 0.0168 = mg/dL
mg/dL × 59.5 = µmol/L
mg/dL × 0.059 = mmol/L

Limitations - interference

Criterion: Recovery within ± 10 % of initial value at an uric acid concentration of 420 µmol/L (7.06 mg/dL).

Serum/plasma

Icterus:[11] No significant interference up to an I index of 39 for conjugated bilirubin and 35 for unconjugated bilirubin (approximate conjugated bilirubin concentration: 667 µmol/L or 39 mg/dL; approximate unconjugated bilirubin concentration: 599 µmol/L or 35 mg/dL).

Hemolysis:[11] No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 621 µmol/L or 1000 mg/dL).

Lipemia (Intralipid):[11] No significant interference up to an L index of 2000. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[12,13] Exceptions: Of the drugs tested in vitro, calcium dobesilate (e.g. Dexium) causes interference at therapeutic concentrations (uric acid level artificially low). Dicynone (Etamsylate) at therapeutic concentrations may lead to false-low results.[14]

Ascorbic acid: No significant interference up to an ascorbic acid level of 1.7 mmol/L (30 mg/dL).

Acetaminophen intoxications are frequently treated with N-Acetylcysteine. N-Acetylcysteine at the therapeutic concentration when used as an antidote and the Acetaminophen metabolite N-acetyl-p-benzoquinone imine (NAPQI) independently may cause falsely low results.

Venipuncture should be performed prior to the administration of Metamizole. Venipuncture immediately after or during the administration of Metamizole may lead to falsely low results.

Uricase reacts specifically with uric acid. Other purine derivatives can inhibit the uric acid reaction.

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[15]

Urine

Drugs: No interference was found at therapeutic concentrations using common drug panels.[13] Exceptions: Levodopa and methyldopa cause interference at therapeutic concentrations (uric acid level artificially low). Dicynone (Etamsylate) at therapeutic concentrations may lead to false-low results.

Ascorbic acid: No significant interference up to an ascorbic acid level of 1.7 mmol/L (30 mg/dL).

Acetaminophen, Acetylcysteine and Metamizole are metabolized quickly. Therefore, interference from these substances is unlikely but cannot be excluded.

High homogentisic acid concentrations in urine samples lead to false results.

Criterion: Recovery within ± 10 % of initial value at an uric acid concentration of 5474 µmol/L (92 mg/dL).

Urea: No significant interference from urea up to a concentration of 2100 mmol/L (12612 mg/dL).

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

ACTION REQUIRED

Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on the **cobas c** 111 analyzer. For information about test combinations requiring special wash steps, please refer to the latest version of the carry-over evasion list found with the CLEAN Method Sheet and the operator's manual for further instructions.

Where required, special wash/carry-over evasion programming must be implemented prior to reporting results with this test.

Limits and ranges

Measuring range

Serum/plasma

12-1500 µmol/L (0.20-25 mg/dL)

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:10 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 10.

Urine

131-16005 µmol/L (2.20-269 mg/dL)

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:10 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 10.

Lower limits of measurement

Serum/plasma

Lower detection limit of the test:
12 µmol/L (0.20 mg/dL)

The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying 3 standard deviations above that of the lowest standard (standard 1 + 3 SD, repeatability, n = 21).

Urine

Lower detection limit of the test:
131 µmol/L (2.20 mg/dL)

The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying 3 standard deviations above that of the lowest standard (standard 1 + 3 SD, repeatability, n = 21).

Expected values

Serum, plasma[16]

Males	202.3-416.5 µmol/L	(3.4-7.0 mg/dL)
Females	142.8-339.2 µmol/L	(2.4-5.7 mg/dL)

Urine (reference range according to Krieg and Colombo)

1st morning urine[17]	2200-5475 µmol/L	(37-92 mg/dL)
24 hours urine[18]	1200-5900 µmol/day	(200-1000 mg/day)
corresponding to	773-3986 µmol/L[b)]	(13-67 mg/dL)

b) calculated from a urine volume of 1.5 L/24 hours

Urine (reference range according Tietz)[19]

Average diet	250-750 mg/24 hours
Low purine diet	
Males	< 480 mg/24 hours
Females	< 400 mg/24 hours
High purine diet	< 1000 mg/24 hours

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

Specific performance data

Representative performance data on the **cobas c** 111 analyzer are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using human samples and controls in an internal protocol with repeatability (n = 21) and intermediate precision (3 aliquots per run, 1 run per day, 10 days). The following results were obtained:

Serum/plasma

Repeatability	*Mean µmol/L (mg/dL)*	*SD µmol/L (mg/dL)*	*CV %*
Precinorm U	248 (4.17)	1 (0.01)	0.3
Precipath U	637 (10.7)	2 (0.0)	0.3
Human serum 1	239 (4.02)	1 (0.02)	0.5
Human serum 2	943 (15.8)	3 (0.1)	0.4

Intermediate precision	*Mean µmol/L (mg/dL)*	*SD µmol/L (mg/dL)*	*CV %*
Precinorm U	253 (4.26)	2 (0.03)	0.8
Precipath U	647 (10.9)	6 (0.1)	0.9
Human serum 3	210 (3.53)	1 (0.02)	0.6
Human serum 4	1004 (16.9)	5 (0.1)	0.5

Urine

Repeatability	*Mean µmol/L (mg/dL)*	*SD µmol/L (mg/dL)*	*CV %*
Control level 1	461 (7.75)	13 (0.22)	2.9
Control level 2	792 (13.3)	12 (0.2)	1.4
Control level 3	1085 (18.2)	17 (0.3)	1.6
Urine sample 1	2263 (38.0)	16 (0.3)	0.7
Urine sample 2	5041 (84.7)	20 (0.3)	0.4
Urine sample 3	12995 (218)	162 (3)	1.3

Method comparison

Uric acid values for human serum, plasma and urine samples obtained on the **cobas c** 111 analyzer (y) were compared with those determined using the corresponding reagent on a COBAS INTEGRA 400 analyzer (x).

Serum/plasma

Sample size (n) = 103

Passing/Bablok[20]	Linear regression
y = 0.992x + 2.15 µmol/L	y = 0.993x + 2.20 µmol/L
τ = 0.987	r = 1.00

The sample concentrations were between 114 and 1465 µmol/L (1.91 and 24.6 mg/dL).

Urine

Sample size (n) = 81

Passing/Bablok[20]	Linear regression
y = 0.952x + 34.9 µmol/L	y = 0.953x + 37.4 µmol/L
τ = 0.989	r = 0.999

The sample concentrations were between 141 and 14157 µmol/L (2.36 and 238 mg/dL).

References

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A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see navifyportal.roche.com for definition of symbols used):

CONTENT	Contents of kit
REAGENT	Reagent
→	Volume for reconstitution
GTIN	Global Trade Item Number
Rx only	For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

UA2

Uric Acid ver.2

Additions, deletions or changes are indicated by a change bar in the margin.

CE 0123

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