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Uric Acid ver.2

Materials provided

REF		CONTENT	Analyzer(s) on which cobas c pack(s) can be used
05171857190*	05171857500	Uric Acid ver.2 (1000 tests)	**cobas** c 701/702
05171857214*	05171857500	Uric Acid ver.2 (1000 tests)	**cobas** c 701/702

* Some kits shown may not be available in all countries.

For reagents, refer to the "Reagents" section.

Materials required (but not provided)

REF	Description	Code
10759350190	Calibrator f.a.s. (12 × 3 mL)	401
05117003190	PreciControl ClinChem Multi 1 (20 × 5 mL)	391
05947626190	PreciControl ClinChem Multi 1 (4 × 5 mL)	391
05117216190	PreciControl ClinChem Multi 2 (20 × 5 mL)	392
05947774190	PreciControl ClinChem Multi 2 (4 × 5 mL)	392
05172152190	Diluent NaCl 9 % (119 mL)	System-ID 08 6869 3
	General laboratory equipment	

System information

Short name	ACN (application code number)	Description
UA2	8700	serum/plasma
UA2-U	8702	urine

Intended use

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

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]

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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.

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

In the presence of peroxidase, 4-aminophenazone is oxidized by hydrogen peroxide to a quinone-diimine dye.

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

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

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
R3	Phosphate buffer: 0.1 mol/L, pH 7.8; potassium 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 µkat/L (25 °C); stabilizers; preservative

R1 is in position B and R3 is in position C.

Warnings and precautions

For in vitro diagnostic use for laboratory 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 safe disposal.

The Safety Data Sheet is available for professional users on request.

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

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 for all countries: +49-621-7590

Storage and stability

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Shelf life at 2-8 °C	See expiration date on **cobas** c pack label.
On-board in use and refrigerated on the analyzer	4 weeks
On-board on the Reagent Manager	24 hours

Calibration

Calibrators	S1: H_2O
	S2: C.f.a.s.
Calibration mode	Linear
Calibration frequency	2-point calibration • after reagent lot change • as required following quality control procedures

The calibration interval may be extended based on acceptable calibration verification values determined by the laboratory.

Traceability: This method has been standardized against ID/MS.[9]

Quality control

Serum/plasma

For quality control, use the control materials listed in the “Materials required (but not provided)” section or other suitable control material.

Urine

Quantitative urine controls are recommended for routine quality control.

Adjust the limits and control intervals based on the laboratory’s individual requirements. If values fall outside the limits, each laboratory is advised to establish corrective measures.

Follow the applicable government regulations and local guidelines.

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 and K2 EDTA plasma.

Specimens derived from capillary blood were found acceptable.[10]

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. 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 urine alkaline (pH > 8.0). To achieve stated uric acid stability, add NaOH prior to sample collection. Urine samples are diluted 1 + 10 with distilled/deionized water or 0.9 % NaCl. This dilution is taken into account in the calculation of the results.

Centrifuge samples containing precipitates before performing the assay.

Refer to the "Limitations and interferences" section for details on possible sample interferences.

Stability in serum/plasma:[11]	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 (upon NaOH addition):[11]	4 days at 20-25 °C

Test procedure

The product is ready for use.

For optimum performance of the assay, follow the instructions given in this document for the corresponding analyzer. For analyzer-specific assay instructions, refer to the corresponding User Guide.

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

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Application for serum and plasma

cobas c 701/702 test definition			
Assay type	2-Point End		
Reaction time / Assay points	10/18-24		
Wavelength (sub/main)	700/546 nm		
Reaction direction	Increase		
Units	mg/dL (µmol/L, mg/L)		
Reagent pipetting		Diluent (H_2O)	
R1	72 µL	25 µL	
R3	14 µL	20 µL	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	3 µL	–	–
Decreased	12 µL	15 µL	135 µL
Increased	6 µL	–	–

Application for urine

cobas c 701/702 test definition			
Assay type	2-Point End		
Reaction time / Assay points	10/18-24		
Wavelength (sub/main)	700/546 nm		
Reaction direction	Increase		
Units	mg/dL (µmol/L, mg/L)		
Reagent pipetting		Diluent (H_2O)	
R1	72 µL	25 µL	
R3 (STAT R2)	14 µL	20 µL	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	3 µL	15 µL	150 µL
Decreased	3 µL	6 µL	160 µL
Increased	6 µL	15 µL	150 µL

Calculation

The **cobas** c systems automatically calculate the analyte concentration of each sample.

Conversion factors: mg/dL × 59.5 = µmol/L
mg/dL × 10.0 = mg/L

Limitations and interferences

Criterion: recovery within ± 10 % of initial value at a uric acid concentration of 7 mg/dL (417 µmol/L) in serum/plasma and at a uric acid concentration of 92 mg/dL (5474 µmol/L) in urine. Recovery within ± 10 % for drug interference.

Serum/plasma

Icterus:[12] no significant interference up to an I index of 40 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 684 µmol/L or 40 mg/dL).

Hemolysis:[12] no significant interference up to an H index of 1000 (approximate hemoglobin concentration: 621 µmol/L or 1000 mg/dL).

Lipemia (Intralipid):[12] no significant interference up to an L index of 1500. 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.[13,14]

Exceptions: Calcium dobesilate causes artificially low uric acid results.

Dicynone (Etamsylate) at therapeutic concentrations may lead to false-low results.[15]

Ascorbic acid: no significant interference from ascorbic acid up to a concentration of 0.17 mmol/L (3 mg/dL).

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

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.

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[16]

Urine

Drugs: No interference was found at therapeutic concentrations using common drug panels.[14]

Exceptions: Calcium dobesilate, Levodopa, and methyldopa can all cause artificially low uric acid results.

Dicynone (Etamsylate) at therapeutic concentrations may lead to false-low results.

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

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

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

For diagnostic purposes, always assess the results 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 **cobas** c systems. All special wash programming necessary for avoiding carryover is available via **cobas** link. In certain cases, manual input is required. The latest version of the carryover evasion list can be found on the NaOHD - SMS - SmpCln1+2 - SCCS Method Sheet. For further instructions, refer to the User Guide.

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

Limits and ranges

Measuring range

Serum/plasma

0.2-25.0 mg/dL (11.9-1487 µmol/L)

Determine samples that have higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:2.5 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 2.5.

Urine

2.2-275 mg/dL (131-16362 µmol/L)

Determine samples that have higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:2.5 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 2.5.

Lower limits of measurement

Serum/plasma

0.2 mg/dL (11.9 µmol/L)

The lower detection limit represents the lowest measurable analyte level that can be distinguished from 0. The lower detection limit is calculated as the value lying 3 standard deviations above the value of the lowest standard (standard 1 + 3 SD, repeatability, n = 21).

Values below the lower detection limit (< 0.2 mg/dL) will not be flagged by the instrument.

Urine

2.2 mg/dL (131 µmol/L)

The lower detection limit represents the lowest measurable analyte level that can be distinguished from 0. The lower detection limit is calculated as the value lying 3 standard deviations above the value of the lowest standard (standard 1 + 3 SD, repeatability, n = 21).

Values below the lower detection limit (< 2.2 mg/dL) will not be flagged by the instrument.

Expected values

Serum/plasma[17]

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

Urine (reference range according to Krieg and Colombo)

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1st morning urine[18]	37-92 mg/dL	(2200-5475 µmol/L)
24-hour urine[19]	200-1000 mg/day	(1200-5900 µmol/day)
corresponding to	13-67 mg/dL	(773-3986 µmol/L)
(calculated from a urine volume of 1.5 L/24 h)		

Urine (reference range according to Tietz)[20]

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

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

Specific performance data

Representative performance data on the analyzers is given below. Results obtained in individual laboratories may differ from the representative performance data.

Precision

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

Serum/plasma

Repeatability	Mean mg/dL (µmol/L)	SD mg/dL (µmol/L)	CV %
Precinorm U	4.29 (255)	0.03 (2)	0.6
Precipath U	10.5 (625)	0.0 (2)	0.3
Human serum A	5.03 (299)	0.03 (2)	0.6
Human serum B	11.1 (660)	0.1 (6)	0.6
Human serum C	22.3 (1327)	0.1 (6)	0.4

Intermediate precision	Mean mg/dL (µmol/L)	SD mg/dL (µmol/L)	CV %
Precinorm U	4.47 (266)	0.07 (4)	1.5
Precipath U	11.1 (660)	0.2 (12)	1.6
Human serum 3	3.96 (236)	0.05 (3)	1.3
Human serum 4	7.17 (427)	0.10 (6)	1.3

Urine

Repeatability	Mean mg/dL (µmol/L)	SD mg/dL (µmol/L)	CV %
Control level 1	7.36 (438)	0.11 (7)	1.4
Control level 2	14.1 (839)	0.1 (6)	0.9
Urine A	44.6 (2654)	0.5 (30)	1.2
Urine B	127 (7557)	1 (60)	0.6
Urine C	223 (13269)	2 (119)	0.7

Intermediate precision	Mean mg/dL (µmol/L)	SD mg/dL (µmol/L)	CV %
Control level 1	11.4 (678)	0.2 (12)	1.9
Control level 2	21.3 (1267)	0.3 (18)	1.6
Urine 3	29.3 (1743)	0.9 (54)	3.0
Urine 4	32.1 (1910)	0.8 (48)	2.3

Results for intermediate precision were obtained on the **cobas** c 501 analyzer.

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Method comparison

Uric acid values for human serum, plasma, and urine samples obtained on a **cobas** c 701 analyzer (y) were compared with values determined using the corresponding reagent on a **cobas** c 501 analyzer (x).

Serum/plasma

Sample size (n) = 96

Passing/Bablok[21]	Linear regression
y = 0.992x – 0.010 mg/dL	y = 1.008x – 0.122 mg/dL
τ = 0.982	r = 1.000

The sample concentrations were between 2.60 and 22.8 mg/dL (155 and 1357 µmol/L).

Urine

Sample size (n) = 110

Passing/Bablok[21]	Linear regression
y = 0.997x – 0.572 mg/dL	y = 1.004x – 0.898 mg/dL
τ = 0.984	r = 1.000

The sample concentrations were between 3.40 and 259 mg/dL (202 and 15411 µmol/L).

Additional information

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

A point (period/stop) is always used in the English version of a Method Sheet as the decimal separator to mark the boundary between the integral and the fractional parts of a decimal numeral. The translated Method Sheets use decimal commas. Labels only use the decimal point as separator. Separators for thousands are not used.

Report any serious incident that has occurred in relation to the device to the manufacturer and the competent authority of the member state in which the user and/or patient is established.

Symbols

In addition to the ISO 15223-1 standard, Roche Diagnostics uses the following symbols and signs:

CONTENT	Contents of kit
→	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.

References

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CE 0123

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Change log

For this document version only:

Due to technical reasons, changes that have been made since the last version of this document are listed in the following table instead of indicated by change bars in the margin.

Section headers are indicated in bold letters.

In addition to the changes listed in the table below, this method sheet version contains several editorial and layout updates.

Section	Current version	Previous version
Materials provided	**Materials provided**	**Order information** **Materials provided**
Materials provided	**Materials provided** without System-ID	**Order information** with System-ID
Materials required (but not provided)	**Materials required (but not provided)**	**Order information** **Materials required (but not provided)**
Materials required (but not provided)	outphased: REF 12149435122 Precinorm U plus REF 12149443122 Precipath U plus	with: REF 12149435122 Precinorm U plus REF 12149443122 Precipath U plus
Reagents	**Reagents**	**Reagents - working solutions**
Warnings and precautions	**Warnings and precautions**	**Precautions and warnings**
Warnings and precautions	laboratory	health care
Specimen collection and preparation	Specimens derived from capillary blood were found acceptable. [Maroto-García J et al.]	
Test procedure	**Test procedure**	**Reagent handling** **Assay**

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Section	Current version	Previous version
Limitations and interferences	**Limitations and interferences**	**Limitations - interference**
Additional information	**Additional information**	
References	Maroto-García J, Deza S, Fuentes-Bullejos P, et al. Analysis of common biomarkers in capillary blood in routine clinical laboratory. Preanalytical and analytical comparison with venous blood. Diagnosis 2023 Mar;10(3):281-297.	