Uric Acid

Order information

COBAS INTEGRA® Uric Acid	500 Tests	Cat. No. 20756296 System-ID 07 5629 6
Calibrator f.a.s.	10 × 3 mL	Cat. No. 10759350 System-ID 07 3718 6
Precinorm® U	20 × 5 mL	Cat. No. 10171743 System-ID 07 7997 0
Precipath® U	20 × 5 mL	Cat. No. 10171778 System-ID 07 7998 9
Precinorm® U plus	10 × 3 mL	Cat. No. 12149435 System-ID 07 7999 7
Precipath® U plus	10 × 3 mL	Cat. No. 12149443 System-ID 07 8000 6
COBAS INTEGRA Cleaner Cassette	150 Tests	Cat. No. 20764337 System-ID 07 6433 7

● Indicates analyzer(s) on which cassette can be used

INTEGRA 400/ 400 plus	INTEGRA/ INTEGRA 700	INTEGRA 800
●	●	●

Intended use
The cassette COBAS INTEGRA Uric Acid (UA) contains an in vitro diagnostic reagent system intended for use on COBAS INTEGRA systems for the quantitative determination of the uric acid concentration in serum, plasma, and urine. This method sheet describes the application for serum, plasma (test UA, 0-029), and urine (test UAU, 0-129).

Summary[1]
Uric acid is the major end product of purine metabolism and is one of the components of the nonprotein nitrogen fraction in plasma. Most uric acid formation occurs in the liver and is derived either from ingested or endogenous nucleoproteins. Approximately half of the total uric acid in the body is eliminated daily by urinary excretion and destruction in the intestinal tract.
Numerous disease states and physiological conditions are associated with alterations in serum uric acid concentrations. Increased levels are more frequent. Serum uric acid levels are characteristically elevated in gout, a disorder involving either uric acid synthesis or excretion.
Other common etiologies of hyperuricemia include renal dysfunction, ketoacidosis, glucose-6-phosphate deficiency, and Lesch-Nyhan syndrome. Decreased uric acid levels have been described in renal tubular absorption defects, Hodgkin's disease, bronchogenic carcinoma, severe hepatocellular disease, and xanthinuria.

Test principle[2,3,4]
Enzymatic colorimetric test with uricase and 4-aminoantipyrine. In the initial step uric acid is oxidized in a reaction catalyzed by uricase. The hydrogen peroxide formed reacts with N-ethyl-N-(2-hydroxy-3-sulfopropyl)-m-toluidine (TOOS) and 4-amino-antipyrine (4-AAP) in the presence of peroxidase (POD) and forms a red quinoneimine dye.

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

$$2\,H_2O_2 + \text{4-AAP} + \text{TOOS} \xrightarrow{\textit{POD}} \text{quinoneimine dye} + 4\,H_2O$$

The color intensity of the quinoneimine formed is directly proportional to the uric acid concentration and is determined by measuring the increase in absorbance at 520 nm.
The addition of ascorbate oxidase prevents interference by ascorbic acid.

Reagents - working solutions
R1 Uricase in vial A (liquid).
R2 Ascorbate oxidase in vial B (liquid).

Active ingredients

Components	Concentrations R1	R2	Test	
Phosphate	120	120	58	mol/L
4-Aminoantipyrine	0.66		0.25	mmol/L
N-ethyl-N-(2-hydroxy-3-sulfopropyl)-m-toluidine	2		0.8	mmol/L
Potassium hexacyanoferrate (II)	100		40	µmol/L
POD (horseradish)	≥100		≥40	µkat/L (≥2.4 kU/L)
Uricase (microbial)	≥5		≥2	µkat/L (≥120 kU/L)
Ascorbate oxidase (cucurbita)		≥100	≥8	µkat/L (≥0.5 kU/L)
pH	7.5	6.1	7.1	

Both reagents contain non-reactive stabilizers.
Please see cassette label for reagent filling volumes.

Precautions and warnings
Pay attention to all precautions and warnings listed in Chapter 1, Introduction.

Reagent handling
Ready for use.

Storage and stability

Shelf life at 2 to 8°C	See expiration date on cassette
INTEGRA 400	
On-board in use at 10 to 15°C	12 weeks
INTEGRA 700/800	
On-board in use at 8°C	8 weeks

Specimen collection and preparation

Only the specimens listed below were tested and found acceptable.
Serum: Collect serum using standard sampling tubes.
Plasma: Li-heparin or EDTA plasma.
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). Urine samples are automatically prediluted 1:10 (1+9) with water by the instrument.
When processing samples in primary tubes, follow the instructions of the tube manufacturer.

Stability in *serum/plasma:*[5]	5 days at 4-8°C
	6 months at -20°C
Stability in *urine:*[6]	
(upon NaOH addition):	4 days at 20-25°C

Centrifuge samples containing precipitates before performing the assay.

Materials provided

See "Reagents - working solutions" section for reagents.

Materials required (but not provided)

COBAS INTEGRA Cleaner Cassette, Cat. No. 20764337, System-ID 07 6433 7. We recommend the use of extra wash cycles when certain test combinations are run together on COBAS INTEGRA systems. For information about test combinations requiring extra wash cycles, please refer to Chapter 1, Introduction, Part III.

Assay

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

Application for serum, plasma and urine

INTEGRA 400 test definition

Measuring mode	Absorbance
Abs. calculation mode	Endpoint
Reaction direction	Increase
Wavelength A/B	520/659 nm
Calc. first/last	T_0/69
Unit	µmol/L
Serum, plasma	
Reaction mode	R1/R2-S
Test range	0-1500 µmol/L (0-25 mg/dL)
with postdilution	0-15 000 µmol/L (0-250 mg/dL)
Postdilution factor	10 recommended
Urine	
Reaction mode	D-R1/R2-S
Test range	0-15 000 µmol/L (0-250 mg/dL)
with postdilution	0-150 000 µmol/L (0-2500 mg/dL)
Predilution factor	10
Postdilution factor	10 recommended

Pipetting parameters

Serum, plasma, urine		Diluent (H_2O)
R1	38 µL	40 µL
R2	20 µL	
Sample	2 µL	20 µL
Total volume	120 µL	

INTEGRA 700/800 test definition

Measuring mode	Absorbance
Abs. calculation mode	Endpoint
Reaction direction	Increase
Wavelength A/B	520/659 nm
Calc. first/last	T_0/98
Unit	µmol/L
Serum, plasma	
Reaction mode	R1-R2-S
Test range	0-1500 µmol/L (0-25 mg/dL)
with postdilution	0-15 000 µmol/L (0-250 mg/dL)
Postdilution factor	10 recommended
Urine	
Reaction mode	D-R1-R2-S
Test range	0-15 000 µmol/L (0-250 mg/dL)
with postdilution	0-150 000 µmol/L (0-2500 mg/dL)
Predilution factor	10
Postdilution factor	10 recommended

Pipetting parameters

Serum, plasma, urine		Diluent (H_2O)
R1	38 µL	40 µL
R2	20 µL	
Sample	2 µL	20 µL
Total volume	120 µL	

Calibration

Calibrator	Calibrator f.a.s. Use deionized water as zero calibrator.
Calibration mode	Linear regression
Calibration replicate	Duplicate recommended
Calibration interval	Each cassette (INTEGRA 700/800) Each cassette and every 6 weeks (INTEGRA 400)

Traceability: This method has been standardized against ID-MS[a].[7]
a) Isotope Dilution Mass Spectrometry

Quality control

Quality control serum, plasma	Precinorm U or Precinorm U plus Precipath U or Precipath U plus
Quality control urine	Quantitative urine controls are recommended for routine quality control.
Control interval	24 hours recommended
Control sequence	User defined
Control after calibration	Recommended

Calculation

COBAS INTEGRA analyzers automatically calculate the analyte concentration of each sample. For more details please refer to Chapter 7, Data Analysis, User Manual (COBAS INTEGRA 700), or to Data analysis in the online Help (COBAS INTEGRA 400/800).

Conversion factor: µmol/L × 0.0168 = mg/dL

Limitations - interference

Criterion: Recovery within ±10% of initial value.
Serum, plasma

Hemolysis	No significant interference up to a hemoglobin level of 0.31 mmol/L (5.0 g/L).
Icterus	No significant interference up to a bilirubin level of 85 µmol/L (5 mg/dL).
Lipemia	No significant interference.
Drugs	Of the drugs tested in vitro, methyldopa and noramidopyrine cause artificially low uric acid values at the tested drug level. Refer to Chapter 1, Introduction for a list of tested drugs and their concentration.
Other	Physiological ascorbic acid concentrations do not interfere with the test. Ascorbic acid levels higher than 170 µmol/L (3.0 mg/dL) decrease the apparent uric acid concentration significantly.
	In very rare cases gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.

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

Expected values

Serum, plasma[8]

Females	<340 µmol/L	(<5.7 mg/dL)
Males (≤65 y)	<420 µmol/L	(<7.0 mg/dL)
Males (>65 y)	<500 µmol/L	(<8.4 mg/dL)

Urine (reference range according to Krieg and Colombo)[9,10]

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

b) Calculated from a urine volume of 1.5 L/24 h

Urine (reference range according to Tietz)[5]

Average diet	250-750mg/24 hours
Low purine diet	
Females	<400 mg/24 hours
Males	<480 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 for serum and plasma[7]

Representative performance data on the COBAS INTEGRA analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Reproducibility was determined using human samples and controls in an internal protocol (within run n = 20, between run n = 20). The following results were obtained.

	Level 1	Level 2
Mean	340 µmol/L (5.7 mg/dL)	635 µmol/L (10.7 mg/dL)
CV within run	0.65%	0.5%
CV between run	2.1%	1.9%

Analytical sensitivity (lower detection limit)

4.71 µmol/L (0.079 mg/dL)
The detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying three standard deviations above that of a zero sample (zero sample + 3 SD, within run precision, n = 30).

Method comparison

Uric acid values for human serum and plasma samples obtained on COBAS INTEGRA 700 with the cassette COBAS INTEGRA Uric Acid were compared to those determined with commercially available reagents for uric acid on COBAS MIRA and an alternative manufacturer's clinical chemistry system. Samples were measured in duplicate. Sample size (n) represents all replicates. Values ranged from 69 to 824 µmol/L (1.2 to 13.8 mg/dL).

		COBAS MIRA	Alternative system
Sample size	(n)	240	240
Corr. coefficient	(r)	0.990	0.996
	(r_s)	0.991	0.994
Lin. regression		$y = 1.00x + 1.0$ µmol/L	$y = 1.04x - 24$ µmol/L
Passing Bablok		$y = 1.00x + 0.8$ µmol/L	$y = 1.04x - 24$ µmol/L

Specific performance data for urine[7]

Representative performance data on the COBAS INTEGRA analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Reproducibility was determined using human samples and controls in an internal protocol (within run n = 20, between run n = 20). The following results were obtained.

	Level 1	Level 2
Mean	1.64 mmol/L (27.6 mg/dL)	3.86 mmol/L (64.8 mg/dL)
CV within run	1.0%	0.67%
CV between run	1.7%	1.2%

Analytical sensitivity (lower detection limit)

0.095 mmol/L (1.6 mg/dL)
The detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying three standard deviations above that of a zero sample (zero sample + 3 SD, within run precision, n = 30).

Method comparison

Uric acid values for human urine samples obtained on COBAS INTEGRA 700 with the cassette COBAS INTEGRA Uric Acid were compared to those determined with a commercially available reagent for uric acid on an alternative manufacturer's clinical chemistry system. Samples were measured in duplicate. Sample size (n) represents all replicates.
Values ranged from 0.20 to 3.68 mmol/L (3.36 to 61.8 mg/dL).

		Alternative system
Sample size	(n)	136
Corr. coefficient	(r)	0.995
	(r_s)	0.993
Lin. regression		$y = 1.15x - 0.09$ mmol/L
Passing Bablok		$y = 1.15x - 0.08$ mmol/L

References

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