UREAL

cobas®

Urea/BUN

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
04460715 190	Urea/BUN 500 tests	System-ID 07 6303 9	Roche/Hitachi **cobas c** 311, **cobas c** 501/502
10759350 190	Calibrator f.a.s. (12 x 3 mL)	Code 401	
12149435 122	Precinorm U plus (10 x 3 mL)	Code 300	
12149443 122	Precipath U plus (10 x 3 mL)	Code 301	
10171743 122	Precinorm U (20 x 5 mL)	Code 300	
10171735 122	Precinorm U (4 x 5 mL)	Code 300	
10171778 122	Precipath U (20 x 5 mL)	Code 301	
10171760 122	Precipath U (4 x 5 mL)	Code 301	
05117003 190	PreciControl ClinChem Multi 1 (20 x 5 mL)	Code 391	
05947626 190	PreciControl ClinChem Multi 1 (4 x 5 mL)	Code 391	
05117216 190	PreciControl ClinChem Multi 2 (20 x 5 mL)	Code 392	
05947774 190	PreciControl ClinChem Multi 2 (4 x 5 mL)	Code 392	
04489357 190	Diluent NaCl 9 % (50 mL)	System-ID 07 6869 3	

English

System information

For **cobas c** 311 analyzer:

UREAL: ACN 418 (serum/plasma)

U-BUN: ACN 421 (serum/plasma)

URELU: ACN 417 (urine)

UBUNU: ACN 428 (urine)

SUREA: ACN 419 (STAT, reaction time: 4, serum/plasma)

SUBUN: ACN 427 (STAT, reaction time: 4, serum/plasma)

SUREU: ACN 420 (STAT, reaction time: 4, urine)

SBUNU: ACN 429 (STAT, reaction time: 4, urine)

For **cobas c** 501 analyzer:

UREAL: ACN 418 (serum/plasma/urine)

U-BUN: ACN 421 (serum/plasma/urine)

SUREA: ACN 419 (STAT, reaction time: 4, serum/plasma/urine)

SUBUN: ACN 427 (STAT, reaction time: 4, serum/plasma/urine)

For **cobas c** 502 analyzer:

UREAL: ACN 8418 (serum/plasma)

U-BUN: ACN 8421 (serum/plasma)

URELU: ACN 8417 (urine)

UBUNU: ACN 8428 (urine)

SUREA: ACN 8419 (STAT, reaction time: 4, serum/plasma)

SUBUN: ACN 8427 (STAT, reaction time: 4, serum/plasma)

SUREU: ACN 8420 (STAT, reaction time: 4, urine)

SBUNU: ACN 8429 (STAT, reaction time: 4, urine)

Intended use

In vitro test for the quantitative determination of urea/urea nitrogen in human serum, plasma and urine on Roche/Hitachi **cobas c** systems.

Summary[1]

Urea is the major end product of protein nitrogen metabolism. It is synthesized by the urea cycle in the liver from ammonia which is produced by amino acid deamination. Urea is excreted mostly by the kidneys but minimal amounts are also excreted in sweat and degraded in the intestines by bacterial action.

Determination of blood urea nitrogen is the most widely used screening test for renal function. When used in conjunction with serum creatinine determinations it can aid in the differential diagnosis of the three types of azotemia: prerenal, renal and postrenal.

Elevations in blood urea nitrogen concentration are seen in inadequate renal perfusion, shock, diminished blood volume (prerenal causes), chronic nephritis, nephrosclerosis, tubular necrosis, glomerular nephritis (renal causes) and urinary tract obstruction (postrenal causes). Transient elevations may also be seen during periods of high protein intake. Unpredictable levels occur with liver diseases.

Test principle

Kinetic test with urease and glutamate dehydrogenase.[2,3,4,5]

Urea is hydrolyzed by urease to form ammonium and carbonate.

$$\text{Urea} + 2\ H_2O \xrightarrow{\text{Urease}} 2\ NH_4^+ + CO_3^{2-}$$

In the second reaction 2-oxoglutarate reacts with ammonium in the presence of glutamate dehydrogenase (GLDH) and the coenzyme NADH to produce L-glutamate. In this reaction two moles of NADH are oxidized to NAD^+ for each mole of urea hydrolyzed.

$$NH_4^+ + \text{2-oxoglutarate} + \text{NADH} \xrightarrow{\text{GLDH}} \text{L-glutamate} + NAD^+ + H_2O$$

The rate of decrease in the NADH concentration is directly proportional to the urea concentration in the specimen and is measured photometrically.

Reagents - working solutions

R1 NaCl 9 %

R2 TRIS buffer: 220 mmol/L, pH 8.6; 2-oxoglutarate: 73 mmol/L; NADH: 2.5 mmol/L; ADP: 6.5 mmol/L; urease (jack bean): ≥ 300 µkat/L; GLDH (bovine liver): ≥ 80 µkat/L; preservative; nonreactive stabilizers

R1 is in position C and R2 is in position B.

Precautions and warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.

Reagent handling

Ready for use

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:	8 weeks

Diluent NaCl 9 %

UREAL

Urea/BUN

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	12 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 and K_2-EDTA plasma. Do not use ammonium heparin.

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

Bacterial growth in the specimen and high atmospheric ammonia concentrations as well as contamination by ammonium ions may cause erroneously elevated results.

Stability in *serum/plasma:*[6]	7 days at 15-25 °C
	7 days at 2-8 °C
	1 year at (-15)-(-25) °C
Stability in *urine:*[6]	2 days at 15-25 °C
	7 days at 2-8 °C
	1 month at (-15)-(-25) °C

Centrifuge samples containing precipitates before performing the assay.

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 and plasma

cobas c 311 **test definition**

Assay type	Rate A		
Reaction time / Assay points	10 / 10-19 (STAT 4 / 10-19)		
Wavelength (sub/main)	700/340 nm		
Reaction direction	Decrease		
Units	mmol/L (mg/dL, g/L)		
Reagent pipetting		Diluent (H_2O)	
R1	10 µL	90 µL	
R2	38 µL	110 µL	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	2 µL	–	–
Decreased	6 µL	15 µL	120 µL
Increased	2 µL	–	–

cobas c 501 **test definition**

Assay type	Rate A		
Reaction time / Assay points	10 / 16-28 (STAT 4 / 16-28)		
Wavelength (sub/main)	700/340 nm		
Reaction direction	Decrease		
Units	mmol/L (mg/dL, g/L)		
Reagent pipetting		Diluent (H_2O)	
R1	10 µL	90 µL	
R2	38 µL	110 µL	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	2 µL	–	–
Decreased	6 µL	15 µL	120 µL
Increased	2 µL	–	–

cobas c 502 **test definition**

Assay type	Rate A		
Reaction time / Assay points	10 / 16-28 (STAT 4 / 16-28)		
Wavelength (sub/main)	700/340 nm		
Reaction direction	Decrease		
Units	mmol/L (mg/dL, g/L)		
Reagent pipetting		Diluent (H_2O)	
R1	10 µL	90 µL	
R2	38 µL	110 µL	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	2 µL	–	–
Decreased	6 µL	15 µL	120 µL
Increased	4 µL	–	–

Application for urine

cobas c 311 **test definition**

Assay type	Rate A		
Reaction time / Assay points	10 / 10-19 (STAT 4 / 10-19)		
Wavelength (sub/main)	700/340 nm		
Reaction direction	Decrease		
Units	mmol/L (mg/dL, g/L)		
Reagent pipetting		Diluent (H_2O)	
R1	10 µL	90 µL	
R2	38 µL	110 µL	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	2 µL	3 µL	147 µL
Decreased	2 µL	2 µL	178 µL
Increased	2 µL	–	–

cobas c 501/502 **test definition**

Assay type	Rate A
Reaction time / Assay points	10 / 16-28 (STAT 4 / 16-28)
Wavelength (sub/main)	700/340 nm
Reaction direction	Decrease
Units	mmol/L (mg/dL, g/L)

UREAL

Urea/BUN

Reagent pipetting		Diluent (H_2O)	
R1	10 µL	90 µL	
R2	38 µL	110 µL	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	2 µL	3 µL	147 µL
Decreased	2 µL	2 µL	178 µL
Increased	2 µL	–	–

Calibration

Calibrators	S1: H_2O S2: C.f.a.s.
Calibration mode	Linear
Calibration frequency	2-point calibration • after 4 weeks on board • after reagent lot change • and as required following quality control procedures

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

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

Roche/Hitachi **cobas c** systems automatically calculate the analyte concentration of each sample.

Conversion factors:	mmol/L urea x 6.006 = mg/dL urea
	mmol/L urea x 0.06006 = g/L urea
	mmol/L urea nitrogen x 2.801 = mg/dL urea nitrogen
	mmol/L urea nitrogen x 0.02801 = g/L urea nitrogen
	mg/dL urea x 0.467 = mg/dL urea nitrogen

When 24-hour urine is used as the specimen, multiply the result by the 24-hour volume to obtain values in g or mmol/24 hours.

Limitations - interference

Criterion: Recovery within ± 10 % of initial value at a urea concentration of 8.3 mmol/L (49.8 mg/dL urea, 23.2 mg/dL urea nitrogen).

Serum/plasma

Icterus:[7] No significant interference up to an I index of 60 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 1026 µmol/L (60 mg/dL)).

Hemolysis:[7] No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 621 µmol/L (1000 mg/dL)).

Lipemia (Intralipid):[7] No significant interference up to an L index of 1000. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Ammonium ions may cause erroneously elevated results.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[8,9]

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[10]

Urine

Drugs: No interference was found at therapeutic concentrations using common drug panels.[9]

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 Roche/Hitachi **cobas c** systems. The latest version of the carry-over evasion list can be found with the NaOHD/SMS/Multiclean/SCCS or the NaOHD/SMS/SmpCln1+2/SCCS Method Sheets. For further instructions refer to the operator's manual. **cobas c** 502 analyzer: All special wash programming necessary for avoiding carry-over is available via the **cobas** link, manual input is not required.

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

0.5-40 mmol/L (3.0-240 mg/dL urea, 1.4-112 mg/dL urea nitrogen)

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:3 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 3.

Urine

1-2000 mmol/L (6-12000 mg/dL urea, 2.8-5600 mg/dL urea nitrogen)

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:1.8 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 1.8.

Determine samples having concentrations lower than the technical limit of 40 mmol/L (240 mg/dL urea and 112 mg/dL urea nitrogen) via the rerun function. Samples are measured undiluted.

Lower limits of measurement

Lower detection limit of the test

Serum/plasma

0.5 mmol/L (3.0 mg/dL urea, 1.4 mg/dL urea nitrogen)

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

1 mmol/L (6 mg/dL urea, 2.8 mg/dL urea nitrogen)

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

Urea:

Serum/plasma[11]

Adults	2.78-8.07 mmol/L	(16.6-48.5 mg/dL)

Urine

24-hour urine[12]	428-714 mmol/24 h (25.7-42.9 g/24 h), corresponding to 286-595 mmol/L (1.71-3.57 g/dL)[a]

a) Based on average urine output of 1.2-1.5 L/24 h

Urea nitrogen (BUN):

Serum/plasma[12]

Adults (18-60 years)	2.14-7.14 mmol/L	6-20 mg/dL

Adults (60-90 years)	2.86-8.21 mmol/L	8-23 mg/dL
Infants (< 1 year)	1.43-6.78 mmol/L	4-19 mg/dL
Infants/children	1.79-6.43 mmol/L	5-18 mg/dL

Urine

24-hour urine[12]	428-714 mmol/24 h (12-20 g/24 h), corresponding to 286-595 mmol/L (801-1666 mg/dL)[b]

b) Based on average urine output of 1.2-1.5 L/24 h

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 analyzers 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 (*serum/plasma:* 3 aliquots per run, 1 run per day, 21 days; *urine:* 3 aliquots per run, 1 run per day, 10 days). The following results were obtained:

Serum/plasma

Repeatability	*Mean*	*SD*	*CV*
	mmol/L (mg/dL urea)	*mmol/L (mg/dL urea)*	*%*
Precinorm U	6.74 (40.5)	0.07 (0.4)	1.0
Precipath U	23.4 (141)	0.2 (1)	0.9
Human serum 1	9.18 (55.1)	0.09 (0.5)	1.0
Human serum 2	15.1 (90.7)	0.1 (0.6)	0.9
Intermediate precision	*Mean*	*SD*	*CV*
	mmol/L (mg/dL urea)	*mmol/L (mg/dL urea)*	*%*
Precinorm U	6.66 (40.0)	0.08 (0.5)	1.2
Precipath U	23.2 (139)	0.3 (2)	1.1
Human serum 3	9.13 (54.8)	0.10 (0.6)	1.1
Human serum 4	14.9 (89.5)	0.2 (1.2)	1.3

Urine

Repeatability	*Mean*	*SD*	*CV*
	mmol/L (mg/dL urea)	*mmol/L (mg/dL urea)*	*%*
Control level 1	161 (967)	4 (24)	2.2
Control level 2	288 (1730)	3 (18)	1.2
Human urine 1	324 (1946)	4 (24)	1.3
Human urine 2	137 (823)	3 (18)	1.9
Intermediate precision	*Mean*	*SD*	*CV*
	mmol/L (mg/dL urea)	*mmol/L (mg/dL urea)*	*%*
Control level 1	154 (925)	4 (24)	2.7
Control level 2	280 (1682)	6 (36)	2.3
Human urine 3	316 (1898)	6 (36)	2.0
Human urine 4	133 (799)	3 (18)	2.4

Method comparison

Urea values for human serum, plasma and urine samples obtained on a Roche/Hitachi **cobas c** 501 analyzer (y) were compared with those determined on Roche/Hitachi 917/MODULAR P analyzers (x), using the corresponding Roche/Hitachi reagent.

Serum/plasma

Sample size (n) = 175

Passing/Bablok[13]	Linear regression
y = 0.990x + 0.138 mmol/L	y = 0.976x + 0.303 mmol/L
τ = 0.959	r = 0.998

The sample concentrations were between 2.27 and 39.4 mmol/L (13.6 and 237 mg/dL urea).

Urine

Sample size (n) = 267

Passing/Bablok[13]	Linear regression
y = 1.006x - 6.50 mmol/L	y = 1.035x - 14.1 mmol/L
τ = 0.949	r = 0.998

The sample concentrations were between 39.0 and 1314 mmol/L (234 and 7892 mg/dL urea).

References

1 Rock RC, Walker WG, Jennings CD. Nitrogen metabolites and renal function. In: Tietz NW, ed. Fundamentals of Clinical Chemistry. 3rd ed. Philadelphia: WB Saunders 1987;669–704.

2 Richterich R, Colombo JP. Klinische Chemie. 4th ed. Basel: Karger S 1978:319-324.

3 Talke H, Schubert GA. Enzymatische Harnstoffbestimmung in Blut und Serum im optischen Test nach Warburg. Klin Wochenschr 1965;43:174.

4 Tiffany TO, Jansen JM, Burtis CA, et al. Enzymatic kinetic rate and end-point analyses of substrate, by use of a GeMSAEC Fast Analyzer. Clin Chem 1972;18:829-840.

5 Sampson EJ, Baired MA, Burtis CA, et al. A coupled-enzyme equilibrium method for measuring urea in serum: Optimization and evaluation of the AACC study group on urea candidate reference method. Clin Chem 1980;26:816-826.

6 WHO Publication: Use of anticoagulants in diagnostic laboratory investigations, WHO/DIL/LAB/99.1 Rev.2:Jan 2002

7 Glick MR, Ryder KW, Jackson SA. Graphical Comparisons of Interferences in Clinical Chemistry Instrumentation. Clin Chem 1986;32:470-475.

8 Breuer J. Report on the Symposium “Drug effects in Clinical Chemistry Methods”. Eur J Clin Chem Clin Biochem 1996;34:385-386.

9 Sonntag O, Scholer A. Drug interference in clinical chemistry: recommendation of drugs and their concentrations to be used in drug interference studies. Ann Clin Biochem 2001;38:376-385.

10 Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. Clin Chem Lab Med 2007;45(9):1240-1243.

11 Löhr B, El-Samalouti V, Junge W, et al. Reference Range Study for Various Parameters on Roche Clinical Chemistry Analyzers. Clin Lab 2009;55:465-471.

12 Wu AHB, ed. Tietz Clinical Guide to Laboratory Tests, 4th edition. St. Louis (MO): Saunders Elsevier 2006;1096.

13 Bablok W, Passing H, Bender R, et al. A general regression procedure for method transformation. Application of linear regression procedures for method comparison studies in clinical chemistry, Part III. J Clin Chem Clin Biochem 1988 Nov;26(11):783-790.

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.

UREAL

Urea/BUN

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Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard.

CONTENT	Contents of kit
→	Volume after reconstitution or mixing
GTIN	Global Trade Item Number

CE

Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
www.roche.com

Roche