0103333825190c501V14.0

TPUC3

Total Protein Urine/CSF Gen. 3

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
03333825 190	Total Protein Urine/CSF Gen.3 (150 tests)	System-ID 07 6763 8	Roche/Hitachi **cobas c** 311, **cobasc** 501/502

Materials required (but not provided):

03121305 122	C.f.a.s. PUC (5 x 1 mL)	Code 489	
03121313 122	Precinorm PUC (4 x 3 mL)	Code 240	
03121291 122	Precipath PUC (4 x 3 mL)	Code 241	
04489357 190	Diluent NaCl 9 % (50 mL)	System-ID 07 6869 3	

English

System information

For **cobas c** 311/501 analyzers:

TPU3: ACN 708

TPC3: ACN 402

For **cobas c** 502 analyzer:

TPU3: ACN 8708

TPC3: ACN 8402

Intended use

In vitro test for the quantitative determination of protein in human urine and cerebrospinal fluid on Roche/Hitachi **cobas c** systems.

Summary

Protein measurements in urine are used in the diagnosis and treatment of disease conditions such as renal or heart diseases, or thyroid disorders, which are characterized by proteinuria or albuminuria. Cerebrospinal fluid (CSF) protein measurements are used in the diagnosis and treatment of conditions such as meningitis, brain tumors and infections of the central nervous system.[1]

Urine is formed by ultrafiltration of plasma across the glomerular capillary wall. Proteins with a relative molecular mass > 40000 are almost completely retained, while smaller substances easily enter the glomerular filtrate. Most CSF protein originates by diffusion from plasma across the blood-CSF barrier. Elevated levels occur as a result of increased permeability of the blood-CSF barrier or with increased local synthesis of immunoglobulins.

Turbidimetric methods using trichloroacetic acid (TCA) or sulfosalicylic acid (SSA) precipitate proteins in the sample depending on their size; the resulting turbidity may be unstable and flocculate. Reagents of dye-binding methods such as Coomassie blue and pyrogallol red-molybdate react with proteins depending on their amino acid composition, but may stain glass and plastic ware. Due to their reaction mechanisms all methods, turbidimetric and colorimetric, exhibit different sensitivities to various proteins, especially to protein fragments such as Bence Jones proteins[2] and small proteins such as α1-microglobulin.

The Roche Diagnostics Urinary/CSF Protein assay is based on the method described by Iwata and Nishikaze,[3] later modified by Luxton, Patel, Keir, and Thompson.[4] In this method, benzethonium chloride reacts with protein in a basic medium to produce a turbidity that is more stable and evenly distributed than that observed with the SSA or TCA methodologies. This assay shows an underrecovery of γ-globulin compared to albumin of about 30 %,[5] and no interference from magnesium ions due to the addition of EDTA.

Test principle

Turbidimetric method.

The sample is preincubated in an alkaline solution containing EDTA, which denatures the protein and eliminates interference from magnesium ions. Benzethonium chloride is then added, producing turbidity.

Reagents - working solutions

R1 Sodium hydroxide: 677 mmol/L; EDTA-Na: 74 mmol/L

R2 Benzethonium chloride: 32 mmol/L

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

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:

Danger

H290	May be corrosive to metals.
H314	Causes severe skin burns and eye damage.
H412	Harmful to aquatic life with long lasting effects.

Prevention:

P273	Avoid release to the environment.
P280	Wear protective gloves/ protective clothing/ eye protection/ face protection/ hearing protection.

Response:

P301 + P330 + P331	IF SWALLOWED: Rinse mouth. Do NOT induce vomiting.
P303 + P361 + P353	IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water.
P304 + P340 + P310	IF INHALED: Remove person to fresh air and keep comfortable for breathing. Immediately call a POISON CENTER/ doctor.
P305 + P351 + P338 + P310	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER/ doctor.

Product safety labeling follows EU GHS guidance.

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

Reagent handling

Ready for use

Storage and stability

TPUC3

Shelf life at 15-25 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	6 weeks

Diluent NaCl 9 %

TPUC3

Total Protein Urine/CSF Gen. 3

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.

Urine

Use random or 24-hour urine specimens. Use no preservatives. Refrigerate specimen during collection.

CSF

No special additives are required. Blood in a CSF specimen invalidates the protein value.[1]

Samples for urinary/CSF protein should be collected before fluorescein is given or at least 24 hours later.[6]

Note: Urine, CSF and control samples with a protein concentration above 7000 mg/L must not be measured with TPUC3 as this may clog the instrument lines.

Stability:[7]

Urine:	1 day at 15-25 °C
	7 days at 2-8 °C
	1 month at (-15)-(-25) °C
CSF:	1 day at 15-25 °C
	6 days at 2-8 °C
	> 1 year at (-15)-(-25) °C

Centrifuge samples containing precipitates before performing the assay.

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

Non centrifuged samples may produce elevated results.

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 urine and CSF

cobas c 311 **test definition**

Assay type	2-Point End		
Reaction time / Assay points	10 / 6-14		
Wavelength (sub/main)	700/505 nm		
Reaction direction	Increase		
Units	mg/L (mg/dL, g/L)		
Reagent pipetting		Diluent (H_2O)	
R 1	100 µL	–	
R 2	40 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluentt (NaCl)*
Normal	6 µL	–	–
Decreased	2 µL	–	–
Increased	6 µL	–	–

cobas c 501 **test definition**

Assay type	2-Point End		
Reaction time / Assay points	10 / 10-30		
Wavelength (sub/main)	700/505 nm		
Reaction direction	Increase		
Units	mg/L (mg/dL, g/L)		
Reagent pipetting		Diluent (H_2O)	
R1	100 µL	–	
R2	40 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	6 µL	–	–
Decreased	2 µL	–	–
Increased	6 µL	–	–

cobas c 502 **test definition**

Assay type	2-Point End		
Reaction time / Assay points	10 / 10-30		
Wavelength (sub/main)	700/505 nm		
Reaction direction	Increase		
Units	mg/L (mg/dL, g/L)		
Reagent pipetting		Diluent (H_2O)	
R1	100 µL	–	
R2	40 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	6 µL	–	–
Decreased	2 µL	–	–
Increased	12 µL	–	–

Calibration

Calibrators	S1: H_2O
	S2-S6: C.f.a.s. PUC
	Multiply the lot-specific C.f.a.s. PUC calibrator values by the factors given below to determine the standard concentrations for the 6-point calibration curve.
	S2: 0.025 S5: 0.250
	S3: 0.050 S6: 1.0
	S4: 0.125
Calibration mode	RCM
Calibration frequency	Full calibration
	- after reagent lot change - as required following quality control procedures

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

Traceability:[8] This method has been standardized against a primary standard traceable to NIST.

Quality control

For quality control, use control materials as listed in the "Order information" section.

In addition, other suitable control material can be used.

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:	mg/L x 0.1 = mg/dL
	mg/L x 0.001 = g/L

To calculate 24-hour urine protein excretion:

mg/L x total volume (liters per 24 hours) = mg/day.

Limitations - interference

Criterion: Recovery within ± 10 % of initial value at a total protein concentration of 120 mg/L (12 mg/dL; 0.12 g/L).

High dose hook-effect: Sample results with high total protein concentrations above the measuring range up to 100000 mg/L will be flagged by the instrument with >TEST or >ABS.

Urine

Icterus: No significant interference up to a conjugated bilirubin concentration of 342 µmol/L or 20 mg/dL.

Hemolysis: Hemoglobin interferes.[9]

Urea: No significant interference from urea up to a concentration of 1300 mmol/L (7809 mg/dL).

Drugs: No interference was found at therapeutic concentrations using common drug panels.[10]

Exception: Levodopa, methyldopa and Na_2-cefoxitin cause artificially high total protein results and calcium dobesilate causes artificially low protein results.

Other: Patient samples containing > 8 g/L of organically bound iodine from Radiopaque media (e.g. Hexabrix) may have falsely elevated results.

High levels of homogentisic acid can be found in urine of patients with the rare genetic disorder Alkaptonuria.[11] Homogentisic acid in urine samples at concentrations > 0.6 mmol/L can cause false results.

The administration of gelatin-based plasma replacements can lead to increased urine protein values.

CSF

Criterion: Recovery within ± 10 % of initial value at a total protein concentration of 450 mg/L.

Hemolysis: Hemoglobin interferes.[9]

Ditaurobilirubin: No significant interference from ditaurobilirubin up to an approximate concentration of 255 µmol/L (15 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 Roche/Hitachi **cobas c** systems. The latest version of the carry-over evasion list can be found with 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 required in certain cases.

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

Limits and ranges

Measuring range

40-2000 mg/L

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.

Lower limits of measurement

Lower detection limit of the test

40 mg/L (4 mg/dL; 0.04 g/L)

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

Urine:[12]	24 h:	< 140 mg/24 h*
	random:	< 150 mg/L*
	* Values obtained from centrifuged samples	
CSF:	reference range acc. to Tietz: 150-450 mg/L (15-45 mg/dL)[13]	
	reference range acc. to Thomas: 200-400 mg/L (20-40 mg/dL)[14]	

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 (3 aliquots per run, 1 run per day, 21 days). The following results were obtained:

Urine

Repeatability	*Mean*	*SD*	*CV*
	mg/L (mg/dL)	*mg/L (mg/dL)*	*%*
Precinorm PUC	159 (15.9)	1 (0.1)	0.7
Precipath PUC	1576 (158)	8 (0.8)	0.5
Human urine 1	101 (10.1)	1 (0.1)	1.0
Human urine 2	191 (19.1)	4 (0.4)	2.2

Intermediate precision	*Mean*	*SD*	*CV*
	mg/L (mg/dL)	*mg/L (mg/dL)*	*%*
Precinorm PUC	156 (15.6)	2 (0.2)	1.5
Precipath PUC	1482 (148)	8 (0.8)	0.5
Human urine 3	106 (10.6)	2 (0.2)	1.6
Human urine 4	154 (15.4)	1 (0.1)	0.9

CSF

Repeatability	*Mean*	*SD*	*CV*
	mg/L (mg/dL)	*mg/L (mg/dL)*	*%*
Control Level 1	281 (28.1)	4 (0.4)	1.5
Control Level 2	691 (69.1)	4 (0.4)	0.6
Human CSF 1	355 (35.5)	4 (0.4)	1.1
Human CSF 2	517 (51.7)	5 (0.5)	1.0

TPUC3

Total Protein Urine/CSF Gen. 3

Intermediate precision	*Mean*		*SD*		*CV*
	mg/L	*(mg/dL)*	*mg/L*	*(mg/dL)*	*%*
Control Level 1	272	(27.2)	4	(0.4)	1.6
Control Level 2	660	(66.0)	6	(0.6)	0.9
Human CSF 3	349	(34.9)	4	(0.4)	1.2
Human CSF 4	501	(50.1)	7	(0.7)	1.5

The data obtained on **cobas c** 501 analyzer(s) are representative for **cobas c** 311 analyzer(s).

Method comparison

Total protein values for human urine and CSF samples obtained on a Roche/Hitachi **cobas c** 501 analyzer (y) were compared with those determined using the corresponding reagent on a Roche/Hitachi 917 analyzer (x).

Urine

Sample size (n) = 70

Passing/Bablok[15]	Linear regression
y = 0.985x + 6.23 mg/L	y = 0.988x + 5.35 mg/L
τ = 0.970	r = 1.000

The sample concentrations were between 47.0 and 1887 mg/L (4.70 and 189 mg/dL).

CSF

Sample size (n) = 86

Passing/Bablok[15]	Linear regression
y = 1.015x - 7.51 mg/L	y = 1.010x - 5.23 mg/L
τ = 0.975	r = 0.999

The sample concentrations were between 53.0 and 1087 mg/L (5.30 and 109 mg/dL).

The data obtained on **cobas c** 501 analyzer(s) are representative for **cobas c** 311 analyzer(s).

References

1 Tietz NW. Fundamentals of Clinical Chemistry, 3rd ed. Pa: WB Saunders Co 1987:336.

2 Boege F. Bence Jones-Proteine. J Lab Med 1999;23(9):477-482.

3 Iwata J, Nishikaze O. New micro-turbidimetric method for determination of protein in cerebrospinal fluid and urine. Clin Chem 1979;25(7):1317-1319.

4 Luxton RW, Patel P, Keir G, et al. A micro-method for measuring total protein in cerebrospinal fluid by using benzethonium chloride in microtiter plate wells. Clin Chem 1989;35(8):1731-1734.

5 Hohnadel DC, Koller A. Urine protein total. In: Pesce AJ, Kaplan LA, editors. Methods in clinical chemistry, St. Louis, Mosby 1987.

6 Koumantakis G. Fluorescein Interference with Urinary Creatinine and Protein Measurements. Clin Chem 1991;37/10:1799.

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

8 Standard Reference Materials from NERL, traceable to NIST (National Institute of Standards and Technology).

9 Yilmaz FM, Yücel D. Effect of Addition of Hemolysate on Urine and Cerebrospinal Fluid Assays for Protein. Clin Chem 2006;52:152-153.

10 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.

11 Phornphutkul C, Introne WJ, Perry MB, et al. Natural History of Alkaptonuria. N Engl J Med 2002;347(26):2111-2121.

12 Junge W, Wilke B, Halabi A, et al. Reference Intervals forTotal Protein in Collected and Random Urine using the Benzethonium Chloride Method [Abstract]. Clin Chem 2006;52:157.

13 Tietz NW, ed. Clinical Guide to Laboratory Tests, 3rd ed. Philadelphia, PA: WB Saunders Company 1995;518-523.

14 Thomas L. Labor und Diagnose, 6. Auflage, TH-Books Verlagsgesellschaft mbH, Frankfurt/Main 2005;930-934.

15 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.

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 dialog.roche.com for definition of symbols used):

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

CE 0123

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