TPUC3

Total Protein Urine/CSF Gen.3

Order information

REF		CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
08058679190*	08058679500	Total Protein Urine/CSF Gen.3 (650 tests)	System-ID 2112 001	**cobas c** 303, **cobas c** 503, **cobas c** 703
08058679214*	08058679500	Total Protein Urine/CSF Gen.3 (650 tests)	System-ID 2112 001	**cobas c** 303, **cobas c** 503, **cobas c** 703

Materials required (but not provided):

03121305122	C.f.a.s. PUC (5 x 1 mL)	Code 20489	
03121313122	Precinorm PUC (4 x 3 mL)	Code 20240	
03121291122	Precipath PUC (4 x 3 mL)	Code 20241	
08063494190	Diluent NaCl 9 % (123 mL)	System-ID 2906 001	

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

English

System information

TPU3: ACN 21122 (Urine)

TPC3: ACN 21123 (CSF)

Intended use

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

Summary

Protein measurements in human urine with this assay are used in the diagnosis and monitoring of disease conditions characterized by proteinuria or albuminuria, including renal or heart diseases, or thyroid disorders.

Protein measurements in human cerebrospinal fluid (CSF) with this assay are used to assess functional blood-brain barriers disorders in case of conditions such as meningitis, brain tumors and infections of the central nervous system.

During glomerular filtration proteins larger than albumin (66 kDa, diameter 3.5 nm, charge -23) are retained by the healthy glomerulus. Proteins with a lower molecular weight are usually able to freely pass through the glomerular membrane but are actively reabsorbed within the tubular system. Therefore, the presence of significant amounts of protein in the urine is suggestive of renal disease.[1] In addition to its role as a marker for renal disease and renal disease risk,[2] proteinuria has been shown to be an independent predictor of cardiovascular morbidity and mortality.[3] Thyroid hormones can directly affect kidney function, and impaired renal function can also contribute to thyroid disorders, most likely due to urinary loss of protein-bound thyroid hormone.[4,5] For this reason, hypothyroid patients often present with proteinuria and viceversa chronic kidney disease patients have higher risk of developing hypothyroidism.[6,7] Most CSF constituents (about 80 %) originate by diffusion from plasma across the blood-CSF barrier.[8] The concentration of CSF proteins is influenced by multiple factors most significantly by blood concentration, protein size, blood-CSF barrier integrity, and intrathecal production.[9] Many acute inflammatory diseases of the CNS, including infections and malignancies of the CNS, are characterized by a mild to moderate increase in total protein concentration.[9,10] Low CSF protein levels can occur in conditions such as repeated lumbar puncture or a chronic leak. Additionally, low CSF protein levels are observed in some children (between 6 months and 2 years of age), in cases of acute water intoxication, and in a minority of patients with idiopathic intracranial hypertension.[10]

The Roche Diagnostics Urinary/CSF Protein assay is based on the method described by Iwata and Nishikaze,[11] later modified by Luxton, Patel, Keir, and Thompson.[12] 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 under recovery of γ-globulin compared to albumin of about 30 %,[13] 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

R3 Benzethonium chloride: 32 mmol/L

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

Precautions and warnings

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 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.

Hazardous components:

- sodium hydroxide

Product safety labeling follows EU GHS guidance.

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

Reagent handling

Ready for use

Storage and stability

Shelf life at 15-25 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	26 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.[14]

Samples for urinary/CSF protein should be collected before fluorescein is given or at least 24 hours later.[15]

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:[16]

Urine:	1 day at 15-25 °C
	7 days at 2-8 °C
	1 month at -20 °C (± 5 °C)
CSF:	1 day at 15-25 °C
	6 days at 2-8 °C
	> 1 year at -20 °C (± 5 °C)

Freeze only once.

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.

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

Test definition

Reporting time	10 min		
Wavelength (sub/main)	700/505 nm		
Reagent pipetting		Diluent (H_2O)	
R1	75 µL	–	
R3	30 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	4.5 µL	–	–
Decreased	4.5 µL	25 µL	50 µL
Increased	4.5 µL	–	–

For further information about the assay test definitions refer to the application parameters setting screen of the corresponding analyzer and assay.

Calibration

Application for urine (ACN 21122)

Calibrators	S1: H_2O S2-S6: C.f.a.s. PUC
Calibration mode	Non-linear
Calibration frequency	Full calibration - after reagent lot change - as required following quality control procedures

Application for CSF (ACN 21123)

Calibrators	S1: H_2O S2-S6: C.f.a.s. PUC
Calibration mode	Non-linear
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:[17] 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.

Urine:	Precinorm PUC, Precipath PUC
CSF:	Precinorm PUC, Precipath PUC

The control intervals and limits should be adapted to each laboratory’s individual requirements. It is recommended to perform quality control always after lot calibration and subsequently at least every 26 weeks.

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

cobas c systems automatically calculate the analyte concentration of each sample in the unit mg/L (mg/dL, g/L).

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

High dose hook-effect: No false result without a flag was observed up to a total protein concentration of 100000 mg/L.

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[18]

Urine

Criterion: Recovery within ± 12 mg/L of initial values of samples ≤ 120 mg/L and within ± 10 % for samples > 120 mg/L.

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

Hemolysis: Hemoglobin interferes.[19]

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.[20]

Exception: Levodopa, methyldopa and Na_2-cefoxitin cause artificially high total protein results at the therapeutic drug level. Phenazopyridine and calcium dobesilate cause artificially low protein results at the therapeutic drug level.

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.[21] 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 ± 45 mg/L of initial values of samples ≤ 450 mg/L and within ± 10 % for samples > 450 mg/L.

Icterus: No significant interference up to an I index of 15 for conjugated bilirubin (approximate conjugated bilirubin concentration: 15 mg/dL).

Hemolysis: Hemoglobin interferes.[19]

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 **cobas c** systems. All special wash programming necessary for avoiding carry-over is available via the **cobas** link. The latest version of the carry-over evasion list can be found with the NaOHD/SMS/SCCS Method Sheet. For further instructions, refer to the operator's manual.

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

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank	= 40 mg/L
Limit of Detection	= 40 mg/L
Limit of Quantitation	= 40 mg/L

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 requirements.

The Limit of Blank is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples.

The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).

The Limit of Quantitation is the lowest analyte concentration that can be reproducibly measured with a total error of 20 %. It has been determined using low concentration total protein urine/CSF samples.

Expected values

Urine[22]

24 h:	< 140 mg/24 h*
Random:	< 150 mg/L*

* Values obtained from centrifuged samples

CSF

Reference range acc. to Tietz:[23]	150-450 mg/L
Reference range acc. to Thomas:[24]	200-400 mg/L*

* calculated by unit conversion factor

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. These data represent the performance of the analytical procedure itself.

Results obtained in individual laboratories may differ due to heterogenous sample materials, aging of analyzer components and mixture of reagents running on the analyzer.

Precision

Precision was determined using human samples and controls in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP05-A3 requirements with repeatability (n = 84) and intermediate precision (2 aliquots per run, 2 runs per day, 21 days). Results for repeatability and intermediate precision were obtained on the **cobas c** 503 analyzer.

Urine

Repeatability	*Mean*	*SD*	*CV*
	mg/L	*mg/L*	*%*
Precinorm PUC	202	5.52	2.7
Precipath PUC	1373	5.64	0.4
Human urine 1	80.4	3.52	4.4
Human urine 2	329	5.05	1.5
Human urine 3	486	5.72	1.2
Human urine 4	994	5.36	0.5
Human urine 5	1644	7.46	0.5
Intermediate precision	*Mean*	*SD*	*CV*
	mg/L	*mg/L*	*%*
Precinorm PUC	202	6.14	3.0
Precipath PUC	1373	7.63	0.6
Human urine 1	74.8	3.88	5.2
Human urine 2	329	5.55	1.7
Human urine 3	499	6.94	1.4
Human urine 4	994	7.65	0.8
Human urine 5	1644	10.2	0.6

CSF

TPUC3

Total Protein Urine/CSF Gen.3

Repeatability	*Mean*	*SD*	*CV*
	mg/L	*mg/L*	*%*
Precinorm PUC	242	9.60	4.0
Precipath PUC	1615	9.06	0.6
Human CSF 1	130	9.52	7.3
Human CSF 2	357	7.72	2.2
Human CSF 3	501	7.16	1.4
Human CSF 4	1087	8.56	0.8
Human CSF 5	1715	10.9	0.6

Intermediate precision	*Mean*	*SD*	*CV*
	mg/L	*mg/L*	*%*
Precinorm PUC	242	11.3	4.7
Precipath PUC	1615	10.7	0.7
Human CSF 1	130	9.88	7.6
Human CSF 2	357	9.57	2.7
Human CSF 3	503	8.29	1.6
Human CSF 4	1063	13.0	1.2
Human CSF 5	1715	12.4	0.7

The data obtained on **cobas c** 503 analyzer(s) are representative for **cobas c** 303 analyzer(s) and **cobas c** 703 analyzer(s).

Method comparison

Total protein values for human urine and CSF samples obtained on a **cobas c** 503 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 501 analyzer (x).

Urine

Sample size (n) = 77

Passing/Bablok[25]	Linear regression
y = 0.952x + 19.3 mg/L	y = 0.948x + 24.0 mg/L
τ = 0.983	r = 0.999

The sample concentrations were between 40.8 and 1784 mg/L.

CSF

Sample size (n) = 75

Passing/Bablok[25]	Linear regression
y = 0.968x + 31.3 mg/L	y = 0.954x + 38.6 mg/L
τ = 0.990	r = 1.000

The sample concentrations were between 43.4 and 1890 mg/L.

Total protein values for human urine and CSF samples obtained on a **cobas c** 303 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 501 analyzer (x).

Urine

Sample size (n) = 71

Passing/Bablok[25]	Linear regression
y = 1.012x + 3.81 mg/L	y = 1.011x + 6.01 mg/L
τ = 0.981	r = 0.999

The sample concentrations were between 45.5 and 1879 mg/L.

CSF

Sample size (n) = 77

Passing/Bablok[25]	Linear regression
y = 1.046x + 17.5 mg/L	y = 1.029x + 25.4 mg/L
τ = 0.987	r = 1.000

The sample concentrations were between 89.8 and 1926 mg/L.

Total protein values for human urine and CSF samples obtained on a **cobas c** 703 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 503 analyzer (x).

Urine

Sample size (n) = 72

Passing/Bablok[25]	Linear regression
y = 0.985x - 2.33 mg/L	y = 0.990x - 3.37 mg/L
τ = 0.979	r = 1.000

The sample concentrations were between 41.8 and 1958 mg/L.

CSF

Sample size (n) = 72

Passing/Bablok[25]	Linear regression
y = 0.987x + 7.13 mg/L	y = 0.985x + 8.20 mg/L
τ = 0.983	r = 1.000

The sample concentrations were between 56.2 and 1970 mg/L.

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:

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.

CE 0123

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