TPUC3

cobas®

Total Protein Urine/CSF Gen.3

Substrates

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	COBAS INTEGRA 400 plus

Materials required (but not provided):

03121305 122	C.f.a.s. PUC (5 × 1 mL)	System-ID 07 6755 7	
03121313 122	Precinorm PUC (4 × 3 mL)	System-ID 07 6756 5	
03121291 122	Precipath PUC (4 × 3 mL)	System-ID 07 6757 3	
20756350 322	NaCl Diluent 9 % (6 × 22 mL)	System-ID 07 5635 0	

English

System information

Test TPU3, test ID 0-163 (urine)

Test TPC3, test ID 0-263 (CSF)

Intended use

In vitro test for the quantitative determination of the total protein concentration in urine and cerebrospinal fluid on COBAS INTEGRA 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.

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 Total Protein Urine/CSF Gen.3 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 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 pre-incubated in an alkaline solution containing EDTA, which denatures the protein and eliminates interference from magnesium ions. Benzethonium chloride is then added, producing turbidity that is read at 512 nm.

Reagents - working solutions

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

SR Benzethonium chloride: 32 mmol/L

R1 is in position B and SR 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

Shelf life at 15-25 °C	See expiration date on **cobas c** pack label
On-board in use at 10-15 °C	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.

Cerebrospinal Fluid (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]

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.

Non centrifuged samples may produce elevated results.

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

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

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

NaCl Diluent 9 %, Cat. No. 20756350322, system-ID 07 5635 0 for automatic postdilution and standard serial dilutions. NaCl Diluent 9 % is placed in its predefined rack position and is stable for 4 weeks on-board.

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.

Application for urine and CSF

Test definition

Measuring mode	Absorbance
Abs. calculation mode	Endpoint
Reaction mode	R1-S-SR
Reaction direction	Increase
Wavelength A	512 nm
Calc. first/last	33/40
Unit	mg/L

Pipetting parameters

		Diluent (H_2O)
R1	100 µL	
Sample	10 µL	15 µL
SR	40 µL	
Total volume	165 µL	

Calibration

Calibrator	C.f.a.s. PUC
Calibration mode	logit/log 4
Calibrator dilution ratio	1:1, 1:4, 1:8, 1:20, 1:40, and 0 mg/L performed automatically by the instrument
Calibration replicate	Duplicate recommended
Calibration interval	Each **cobas c** pack and every 43 days, and as required following quality control procedures.

Enter the assigned lot-specific total protein values (urine and/or CSF) of the undiluted calibrator, indicated in the package insert of the calibrator C.f.a.s. PUC.

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

Traceability: This method has been standardized against an internal method traceable to NIST.

Quality control

Reference range	Precinorm PUC
Pathological range	Precipath PUC
Control interval	24 hours recommended
Control sequence	User defined
Control after calibration	Recommended

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

The COBAS INTEGRA 400 plus analyzer automatically calculates the analyte concentration of each sample. For more details, please refer to Data Analysis in the Online Help.

Conversion factor: mg/L × 0.1 = mg/dL

To calculate 24-hours urine protein excretion:
mg/L × total volume (liters per 24 h) = mg/day

Limitations - interference

Urine

Criterion: Recovery within ± 10 % of initial value.

Icterus: No significant interference up to a conjugated bilirubin concentration of 599 µmol/L or 35 mg/dL.

Hemolysis: Hemoglobin interferes.[8]

Drugs: No interference was found at therapeutic concentrations using common drug panels.[9] Exceptions: Levodopa, Methyldopa and Cefoxitin sodium cause interference at therapeutic concentrations (artificially high total protein levels).

Patient samples containing > 6.4 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.[10] Homogentisic acid in urine samples at concentration > 1.2 mmol/L can cause false results.

The administration of gelatin-based plasma replacements can cause increased urinary protein values.

There is no high dose hook effect at protein concentrations up to 100 g/L.

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[11]

It has been demonstrated that the following substances cause no significant interference when added to a normal or pathological human urine pool:

Ammonium chloride	187 mmol/L	(10 g/L)
Citrate	10 mmol/L	(190 mg/dL)
Creatinine	53 mmol/L	(6 g/L)
Glucose	194 mmol/L	(35 g/L)
Magnesium	75 mmol/L	(1.8 g/L)
Oxalate	10 mmol/L	(90 mg/dL)

Phosphate	39 mmol/L	(1.2 g/L)
Uric acid	5 mmol/L	(85 mg/dL)

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

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

CSF

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

Ditaurobilirubin: No significant interference from ditaurobilirubin up to an approximate concentration of 255 µmol/L (15 mg/dL).

Hemolysis: Hemoglobin interferes.[8]

Extremely high samples far outside the measuring range may give false-low results.

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 INTEGRA analyzers. Refer to the CLEAN Method Sheet for further instructions and for the latest version of the Extra wash cycle list.

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

Limits and ranges

Measuring range (urine and CSF)

40-2000 mg/L (4-200 mg/dL)

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)

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 a zero sample (zero sample + 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:[13]	150-450 mg/L (15-45 mg/dL)
reference range acc. to Thomas:[14]	200-400 mg/L (20-40 mg/dL)

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 COBAS INTEGRA 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 (1 aliquot per run, 1 run per day, 21 days). The following results were obtained:

Urine

Repeatability	*Mean mg/L*	*SD mg/L*	*CV %*
Precinorm PUC	245	1	0.5
Precipath PUC	789	3	0.3

Intermediate precision	*Mean mg/L*	*SD mg/L*	*CV %*
Precinorm PUC	329	3	0.9
Precipath PUC	792	3	0.4

CSF

Repeatability	*Mean mg/L*	*SD mg/L*	*CV %*
Precinorm PUC	329	2	0.6
Precipath PUC	789	3	0.3

Intermediate precision	*Mean mg/L*	*SD mg/L*	*CV %*
Precinorm PUC	329	3	0.9
Precipath PUC	792	3	0.4

Method comparison

Urine

Total protein values for human urine samples obtained on a COBAS INTEGRA 800 analyzer with the COBAS INTEGRA Total Protein Urine/CSF Gen.3 reagent (y) were compared with those determined using the corresponding reagent on a Roche/Hitachi 917 analyzer (x) and with the previous reagent (TPU-C) on a COBAS INTEGRA 800 analyzer (x).

Roche/Hitachi 917 analyzer

Sample size (n) = 113

Passing/Bablok[15]	Linear regression
y = 0.981x + 6.61 mg/L	y = 0.970x + 20.9 mg/L
τ = 0.923	r = 0.994
SD (md 95) = 56.4	Sy.x = 29.2

The sample concentrations were between 40 and 1788 mg/L (4.0 and 178.8 mg/dL).

COBAS INTEGRA 800 analyzer

Sample size (n) = 137

Passing/Bablok[15]	Linear regression
y = 0.872x + 22.6 mg/L	y = 0.770x + 35 mg/L
τ = 0.762	r = 0.981
SD (md 95) = 87.9	Sy.x = 35.1

The sample concentrations were between 14 and 1675 mg/L (1.4 and 167.5 mg/dL).

CSF

Total protein values for human CSF samples obtained on a COBAS INTEGRA 800 analyzer with the COBAS INTEGRA Total Protein Urine/CSF Gen.3 reagent (y) were compared with those determined using the corresponding reagent on a Roche/Hitachi MODULAR P analyzer (x) and with the previous reagent (TPU-C) on a COBAS INTEGRA 800 analyzer (x).

Roche/Hitachi MODULAR P analyzer

Sample size (n) = 28

Passing/Bablok[15]	Linear regression
y = 0.976x + 1.23 mg/L	y = 0.981x + 2.01 mg/L
τ = 0.979	r = 0.998
SD (md 95) = 13.3	Sy.x = 8.2

The sample concentrations were between 43 and 952 mg/L (4.3 and 95.2 mg/dL).

COBAS INTEGRA 800 analyzer

Sample size (n) = 24

Passing/Bablok[15]	Linear regression
y = 0.87x + 1.35 mg/L	y = 0.86x - 0.48 mg/L
τ = 0.935	r = 0.996
SD (md 95) = 28.1	Sy.x = 15.4

The sample concentrations were between 171 and 1296 mg/L (17.1 and 129.6 mg/dL).

References

1 Tietz NW. Fundamentals of Clinical Chemistry. 3rd ed. Philadelphia: WB Saunders 1987:336,339-341.

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

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:The C.W. Mosby Co.,1987:35-45.

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

7 Use of Anticoagulants in Diagnostic Laboratory Investigations. WHO Publication WHO/DIL/LAB/99.1 Rev. 2: Jan 2002.

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

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

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

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. Fundamentals of Clinical Chemistry, 3rd ed. Philadelphia, Pa: WB Saunders Co; 1995:520.

14 Thomas L, ed. Labor und Diagnose. 6th ed.: 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

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

CE 0123

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