TP2

Total Protein Gen.2

Order information

REF		CONTENT		Analyzer(s) on which **cobas c** pack (s) can be used
08058652190	08058652500	Total Protein Gen.2 (1050 tests)	System-ID 2111 001	**cobas c** 303, **cobas c** 503

Materials required (but not provided):

10759350190	Calibrator f.a.s. (12 x 3 mL)	Code 20401	
05117003190	PreciControl ClinChem Multi 1 (20 x 5 mL)	Code 20391	
05947626190	PreciControl ClinChem Multi 1 (4 x 5 mL)	Code 20391	
05117216190	PreciControl ClinChem Multi 2 (20 x 5 mL)	Code 20392	
05947774190	PreciControl ClinChem Multi 2 (4 x 5 mL)	Code 20392	
10557897122	Precinorm Protein (3 x 1 mL)	Code 20302	
11333127122	Precipath Protein (3 x 1 mL)	Code 20303	
08063494190	Diluent NaCl 9 % (123 mL)	System-ID 2906 001	

English

System information

TP2: ACN 21110

Intended use

In vitro test for the quantitative determination of total protein in human serum and plasma on **cobas c** systems.

Summary

Measurements of total protein, performed with this assay in human serum or plasma, are used as aid in diagnosis and monitoring of a variety of diseases involving the liver, kidney, or bone marrow, as well as other metabolic or nutritional disorders.[1,2,3,4]

Plasma proteins are synthesized predominantly in the liver, plasma cells, lymph nodes, the spleen and bone marrow. In the course of disease the total protein concentration and also the percentage represented by individual fractions can significantly deviate from normal values. Hypoproteinemia can be caused by diseases and disorders such as loss of blood, sprue, nephrotic syndrome, severe burns, salt retention syndrome and Kwashiorkor (acute protein deficiency).

Hyperproteinemia can be observed in cases of severe dehydration and illnesses such as multiple myeloma. Changes in the relative percentage of plasma proteins can be due to a change in the percentage of one plasma protein fraction. Often in such cases the amount of total protein does not change. The albumin/globulin (A/G) ratio is commonly used as an index of the distribution of albumin and globulin fractions. Marked changes in this ratio can be observed in cirrhosis of the liver, glomerulonephritis, nephrotic syndrome, acute hepatitis, lupus erythematosus as well as in certain acute and chronic inflammations.[1,2,3,4]

Test principle[5]

Colorimetric assay

Divalent copper reacts in alkaline solution with protein peptide bonds to form the characteristic purple-colored biuret complex. Sodium potassium tartrate prevents the precipitation of copper hydroxide and potassium iodide prevents autoreduction of copper.

$$\text{protein} + Cu^{2+} \xrightarrow[\text{solution}]{\text{alkaline}} \text{Cu-protein complex}$$

The color intensity is directly proportional to the protein concentration which can be determined photometrically.

Reagents - working solutions

R1 Sodium hydroxide: 400 mmol/L; potassium sodium tartrate: 89 mmol/L

R3 Sodium hydroxide: 400 mmol/L; potassium sodium tartrate: 89 mmol/L; potassium iodide: 61 mmol/L; copper sulfate: 24.3 mmol/L

R1 is in position B and R3 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:

Warning

H290	May be corrosive to metals.
H315	Causes skin irritation.
H319	Causes serious eye irritation.
H412	Harmful to aquatic life with long lasting effects.

Prevention:

P264	Wash skin thoroughly after handling.
P273	Avoid release to the environment.
P280	Wear protective gloves/ eye protection/ face protection.

Response:

P337 + P313	If eye irritation persists: Get medical advice/attention.
P390	Absorb spillage to prevent material damage.

Disposal:

P501	Dispose of contents/container to an approved waste disposal plant.

Product safety labeling follows EU GHS guidance.

Contact phone: all countries: +49-621-7590, USA: 1-800-428-2336

Reagent handling

Ready for use

TP2

Total Protein Gen.2

cobas®

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.

Serum.

Plasma: Li-heparin and K_2-EDTA plasma

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.

Stability:[6]	6 days at 20-25 °C
	4 weeks at 4-8 °C
	1 year at -20 °C (± 5 °C)

Freeze only once.

The total protein concentration is 4 to 8 g/L lower when the sample is collected from a patient situated in the recumbent position rather than upright.[7]

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

Test definition

Reporting time	10 min		
Wavelength (sub/main)	700/546 nm		
Reagent pipetting		Diluent (H_2O)	
R1	59 µL	18 µL	
R3	21 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	1.3 µL	–	–
Decreased	1.3 µL	25 µL	50 µL
Increased	1.3 µL	–	–

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

Calibration

Calibrators	S1: H_2O
	S2: C.f.a.s.
Calibration mode	Linear
Calibration frequency	Automatic full calibration - after reagent lot change
	Full calibration - as required following quality control procedures

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

Traceability: This method has been standardized against SRM 927.

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. 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 g/L (g/dL).

Conversion factor: g/L x 0.1 = g/dL

Limitations - interference

Criterion: Recovery within ± 10 % of initial value at a total protein concentration of 66 g/L (6.6 g/dL).

Icterus[8]: No significant interference up to an I index of 20 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 342 µmol/L or 20 mg/dL).

Hemolysis[8]: No significant interference up to an H index of 500 (approximate hemoglobin concentration: 311 µmol/L or 500 mg/dL).

Lipemia (Intralipid)[8]: No significant interference up to an L index of 2000. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Dextran: No significant interference from dextran up to a concentration of 30 mg/mL.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[9,10]

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

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

2.0-120 g/L (0.2-12 g/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

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank	= 2.0 g/L (0.2 g/dL)
Limit of Detection	= 2.0 g/L (0.2 g/dL)
Limit of Quantitation	= 3.0 g/L (0.3 g/dL)

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 samples.

Expected values

g/L

Expected values according to Josephson [12]

Adults	66-87 g/L*

* calculated by unit conversion factor

Expected values according to Tietz[13]

Umbilical cord	48-80 g/L
Premature	36-60 g/L
Newborn	46-70 g/L
1 week	44-76 g/L
7 months-1 year	51-73 g/L
1-2 years	56-75 g/L
> 3 years	60-80 g/L
Adults (ambulatory)	64-83 g/L

Expected values according to Australasian Association of Clinical Biochemists[14]

Adults	60-80 g/L

g/dL

Expected values according to Josephson [12]

Adults	6.6-8.7 g/dL

Expected values according to Tietz[13]

Umbilical cord	4.8-8.0 g/dL
Premature	3.6-6.0 g/dL
Newborn	4.6-7.0 g/dL
1 week	4.4-7.6 g/dL
7 months-1 year	5.1-7.3 g/dL
1-2 years	5.6-7.5 g/dL
> 3 years	6.0-8.0 g/dL
Adults (ambulatory)	6.4-8.3 g/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 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.

Repeatability	*Mean*	*SD*	*CV*
	g/L	*g/L*	*%*
PCCC1[a)]	49.5	0.244	0.5
PCCC2[b)]	73.0	0.358	0.5
Human serum 1	4.61	0.140	3.0
Human serum 2	32.1	0.224	0.7
Human serum 3	61.4	0.292	0.5
Human serum 4	72.9	0.394	0.5
Human serum 5	103	0.588	0.6

Intermediate precision	*Mean*	*SD*	*CV*
	g/L	*g/L*	*%*
PCCC1[a)]	49.7	0.396	0.8
PCCC2[b)]	73.0	0.495	0.7
Human serum 1	4.83	0.196	4.1
Human serum 2	32.4	0.326	1.0
Human serum 3	61.4	0.396	0.6
Human serum 4	72.9	0.453	0.6
Human serum 5	103	0.686	0.7

a) PreciControl ClinChem Multi 1

b) PreciControl ClinChem Multi 2

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

Method comparison

Total protein values for human serum 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).

Sample size (n) = 74

Passing/Bablok[15]	Linear regression
y = 1.010x - 0.0180 g/L	y = 1.010x - 0.0639 g/L
τ = 0.975	r = 0.999

The sample concentrations were between 8.43 and 116 g/L.

Total protein values for human serum 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).

Sample size (n) = 74

Passing/Bablok[15]	Linear regression
y = 1.012x - 0.536 g/L	y = 1.015x - 0.785 g/L
τ = 0.979	r = 1.000

The sample concentrations were between 7.50 and 117 g/L.

References

1 Brobeck JR, ed. Physiological Basis of Medical Practice, 9th ed. Baltimore, MD: Wilkins and Wilkins 1973;4-7.

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5 Weichselbaum TE. An accurate and rapid method for the determination of proteins in small amounts of blood serum and plasma. Am J Clin Pathol 1946;10:40-49.

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

12 Josephson B, Gyllenswärd C. The Development of the Protein Fractions and of Cholesterol Concentration in the Serum of Normal Infants and Children. Scandinav J Clin Lab Investigation 1957;9:29.

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

14 Tate JR, Sikaris KA, Jones GRD, et al. Harmonising adult and paediatric reference intervals in Australia and New Zealand: An evidence-based approach for establishing a first panel of chemistry analytes. Clin Biochem Rev 2014; Nov 35(4):213-35.

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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 (for USA: see dialog.roche.com for definition of symbols used):

CONTENT	Contents of kit
→	Volume for reconstitution
GTIN	Global Trade Item Number

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

CE 0123

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