TP2

Total Protein Gen.2

cobas®

Substrates

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
03183734 190	Total Protein Gen.2 (300 tests)	System-ID 07 6827 8	COBAS INTEGRA 400 plus
Materials required (but not provided):			
10759350 190	Calibrator f.a.s. (12 × 3 mL)	System-ID 07 3718 6	
10759350 360	Calibrator f.a.s. (12 × 3 mL, for USA)	System-ID 07 3718 6	
12149435 122	Precinorm U plus (10 × 3 mL)	System-ID 07 7999 7	
12149435 160	Precinorm U plus (10 × 3 mL, for USA)	System-ID 07 7999 7	
12149443 122	Precipath U plus (10 × 3 mL)	System-ID 07 8000 6	
12149443 160	Precipath U plus (10 × 3 mL, for USA)	System-ID 07 8000 6	
10557897 122	Precinorm Protein (3 × 1 mL)	System-ID 07 9105 9	
10557897 160	Precinorm Protein (3 × 1 mL, for USA)	System-ID 07 9105 9	
11333127 122	Precipath Protein (3 × 1 mL)	System-ID 07 9106 7	
11333127 160	Precipath Protein (3 × 1 mL, for USA)	System-ID 07 9106 7	
05117003 190	PreciControl ClinChem Multi 1 (20 × 5 mL)	System-ID 07 7469 3	
05947626 190	PreciControl ClinChem Multi 1 (4 × 5 mL)	System-ID 07 7469 3	
05947626 160	PreciControl ClinChem Multi 1 (4 × 5 mL, for USA)	System-ID 07 7469 3	
05117216 190	PreciControl ClinChem Multi 2 (20 × 5 mL)	System-ID 07 7470 7	
05947774 190	PreciControl ClinChem Multi 2 (4 × 5 mL)	System-ID 07 7470 7	
05947774 160	PreciControl ClinChem Multi 2 (4 × 5 mL, for USA)	System-ID 07 7470 7	

English

System information

Test TP2, test ID 0-027

Intended use

In vitro test for the quantitative determination of the total protein concentration in serum and plasma on COBAS INTEGRA systems.

Summary[1]

Plasma proteins are synthesized predominantly in the liver, plasma cells, lymph nodes, the spleen and in 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 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. Total protein measurements are used in the diagnosis and treatment of a variety of diseases involving the liver, kidney, or bone marrow, as well as other metabolic or nutritional disorders.

Test principle[2]

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.

$$\text{Protein} + Cu^{2+} \xrightarrow{\text{Alkaline pH}} \text{Cu-protein complex}$$

The color intensity is directly proportional to the protein concentration. It is determined by measuring the increase in absorbance at 552 nm.

Reagents - working solutions

R1 Sodium hydroxide: 400 mmol/L; sodium potassium tartrate: 89 mmol/L; pH: 13.4

SR Sodium hydroxide: 400 mmol/L; sodium potassium tartrate: 89 mmol/L; potassium iodide: 61 mmol/L; cupric sulfate: 24.3 mmol/L; pH: 13.2

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.

For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

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.
H411	Toxic 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.
P391	Collect spillage.

Product safety labeling follows EU GHS guidance.

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

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	4 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 or K_3-EDTA plasma.

The total protein concentration is by 0.4-0.8 g/dL lower when the sample is collected from a patient situated in the recumbent position rather than upright.[3]

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.

In rare cases, even correctly handled heparin plasma primary tubes may contain cell aggregates which lead to a specific absorption increase at the secondary wavelength 659 nm and thus to false-low TP results. This interference may be prevented by either transferring the sample supernatant into a secondary tube or, in case of gel tubes, by gently mixing the sample by inverting prior to the analysis.

Centrifuge samples containing precipitates before performing the assay.

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

Stability:[4]	4 weeks at 4-8 °C
	6 days at 20-25 °C
	1 year at –20 °C

Sample stability claims were established by experimental data by the manufacturer or based on reference literature and only for the temperatures/time frames as stated in the method sheet. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine specific stability criteria for its laboratory.

Materials provided

See “Reagents – working solutions” section for reagents.

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 serum and plasma

Test definition

Measuring mode	Absorbance
Abs. calculation mode	Endpoint
Reaction mode	R1-S-SR
Reaction direction	Increase
Wavelength A/B	552/659 nm
Calc. first/last	33/52
Unit	g/L

Pipetting parameters

		Diluent (H_2O)
R1	90 µL	0 µL
Sample	2 µL	28 µL
SR	32 µL	0 µL
Total volume	152 µL	

Calibration

Calibrator	Calibrator f.a.s.
	Use deionized water as zero calibrator.
Calibration mode	Linear regression
Calibration replicate	Duplicate recommended
Calibration interval	Each lot and 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

Reference range	Precinorm U plus, Precinorm Protein or PreciControl ClinChem Multi 1
Pathological range	Precipath U plus, Precipath Protein or PreciControl ClinChem Multi 2
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: g/L × 0.1 = g/dL

Limitations - interference

Criterion: Recovery within ± 10 % of initial value.

Serum/plasma

Icterus:[5] No significant interference up to an I index of 60 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 1026 µmol/L or 60 mg/dL).

Hemolysis:[5] No significant interference up to an H index of 500 (approximate hemoglobin concentration: 310 µmol/L or 500 mg/dL).

Lipemia (Intralipid):[5] 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.[6,7]

Low recovery may be caused by the formation of cell aggregates in some heparin plasma samples.

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[8]

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

2-120 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 1:5 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 5.

Lower limits of measurement

Lower detection limit of the test:
2 g/L (0.2 g/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 = 30).

Expected values

Expected values according to Josephson[9]

Adults	6.6-8.7 g/dL (66-87 g/L)

Expected values according to Tietz[10]

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

Expected values according to Australasian Association of Clinical Biochemists[11]

Adults	60-80 g/L (6.0-8.0 g/dL)

Roche has not evaluated reference ranges in a pediatric population.

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:

Repeatability	*Mean* g/L	*SD* g/L	*CV* %
Human serum 1	70.1	0.8	1.1
Human serum 2	84.2	0.4	0.5
Precinorm U	48.0	0.2	0.5
Precipath U	51.7	0.3	0.5

Intermediate precision	*Mean* g/L	*SD* g/L	*CV* %
Human serum 1	65.4	1.4	2.2
Human serum 2	92.0	1.4	1.5
Precinorm U	52.6	0.5	1.0
Precipath U	51.2	0.9	1.7

Method comparison

Total protein values for human serum samples obtained on a COBAS INTEGRA 700 using the COBAS INTEGRA Total Protein Gen.2 reagent (y) were compared with those determined using the corresponding reagent on a Roche/Hitachi 917 analyzer (x) and with those determined using the previous reagent (TP) on a COBAS INTEGRA 700 analyzer (x).

Roche/Hitachi 917 analyzer

Sample size (n) = 114

Passing/Bablok[12]	Linear regression
y = 0.979x + 0.249 g/L	y = 0.978x + 0.452 g/L
τ = 0.947	r = 0.998
SD (md 95) = 1.54	Sy.x = 0.732

The sample concentrations were between 32 and 100 g/L (3.2 and 10.0 g/dL).

COBAS INTEGRA 700 analyzer

Sample size (n) = 60

Passing/Bablok[12]	Linear regression
y = 1.033x - 0.541 g/L	y = 1.031x - 0.372 g/L
τ = 0.972	r = 0.999
SD (md 95) = 0.984	Sy.x = 0.467

The sample concentrations were between 24 and 113 g/L (2.4 and 11.3 g/dL).

References

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

2 Weichselbaum TE. Amer J Clin Path 1946;16:40.

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

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

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

7 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.

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

9 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.

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

11 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.

12 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

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Roche Diagnostics warrants that this product will meet the specifications stated in the labeling when used in accordance with such labeling and will be free from defects in material and workmanship until the expiration date printed on the label. THIS LIMITED WARRANTY IS IN LIEU OF ANY OTHER WARRANTY, EXPRESS OR IMPLIED, INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR PARTICULAR PURPOSE. IN NO EVENT SHALL ROCHE DIAGNOSTICS BE LIABLE FOR INCIDENTAL, INDIRECT, SPECIAL OR CONSEQUENTIAL DAMAGES.

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