ALBT2

cobas®

Tina-quant Albumin CSF

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
04469658 190	Tina-quant Albumin Gen.2 (100 tests)	System-ID 07 6743 3	Roche/Hitachi **cobas c** 501/502
03121305 122	C.f.a.s. PUC (5 x 1 mL)	Code 489	
03121291 122	Precipath PUC (4 x 3 mL)	Code 241	
05117003 190	PreciControl ClinChem Multi 1 (20 x 5 mL)	Code 391	
05947626 190	PreciControl ClinChem Multi 1 (4 x 5 mL)	Code 391	
05117216 190	PreciControl ClinChem Multi 2 (20 x 5 mL)	Code 392	
05947774 190	PreciControl ClinChem Multi 2 (4 x 5 mL)	Code 392	
04489357 190	Diluent NaCl 9 % (50 mL)	System-ID 07 6869 3	

English

System information

For **cobas c** 501 analyzer:

ALB-C: ACN 440

For **cobas c** 502 analyzer:

ALB-C: ACN 8440

Intended use

In vitro test for the quantitative determination of albumin in human cerebrospinal fluid and corresponding human serum/plasma on Roche/Hitachi **cobas c** 501/502 systems.

Summary[1,2,3,4,5,6,7]

Cerebrospinal fluid (CSF) analysis is a basic tool for diagnosis of neurological diseases.
The diffusion of proteins through the blood-brain barrier normally occurs at a steady rate. The rate is influenced by the permeability of the blood-brain barrier and CSF flow rate. Changes in protein concentration in the CSF can be an indication for various neurological diseases.
Disease-related immunoglobulin patterns (IgG, IgA, IgM with reference to albumin) allow for the differential diagnosis of neurological disorders with the aid of Reiber quotient schemes.
Elevated levels of IgG, IgA, IgM in CSF are often associated with opportunistic infections of the central nervous system (CNS) and neurotuberculosis. Increased CSF IgG, IgA, IgM concentrations may occur because of either increased permeability of the blood-brain barrier or local/intrathecal production of IgG, IgA, IgM or both. Malfunction of the blood-brain barrier can be reliably quantified by means of the albumin CSF/serum ratio.
Albumin is an ideal reference protein for blood-brain barrier function, since it is solely synthesized outside the brain and thereby provides an excellent measure for proteins passing the blood-brain barrier. An elevated albumin CSF/serum ratio is an indication of disorders of the blood-brain barrier.
Measuring IgG, IgA, IgM and albumin in CSF/serum pairs, a differentiation between IgG, IgA, IgM originating from blood and IgG, IgA, IgM originating from intrathecal production is possible. The results of the CSF/serum ratio for IgG, IgA, IgM and Albumin, in conjunction with Reiber quotient scheme provide an aid in the diagnosis of functional blood-brain barriers disorders and/or intrathecal IgG, IgA, IgM synthesis.
Blood brain barrier disorders can be reliably quantified with the aid of the albumin CSF/serum ratio. Elevated albumin ratios are indicative of a blood-brain barrier disorder.
By simultaneously determining IgG, IgA, IgM in CSF and serum while taking into account the individual albumin ratios, it is possible to differentiate between IgG, IgA, IgM originating from the blood and CNS-synthesized immunoglobulin.
Albumin is a non-glycosylated protein with a molecular weight of 66000 Da. It is synthesized in liver parenchymal cells at a rate of 14 g/day.
Quantitatively, albumin is normally the most important protein component (> 50 %) in plasma, CSF and urine.
Albumin has two main functions in plasma: maintaining the oncotic pressure (80 % due to albumin in plasma) and transport. It is the most important transport protein for substances having low water solubility (such as free fatty acids, bilirubin, metal ions, hormones and pharmaceuticals).
Depressed albumin levels are caused by hyperhydration, hepatocellular synthesis insufficiency, secretion disorders in the intravascular space, abnormal distribution between the intravascular and extravascular space, catabolism and loss of albumin, acute phase reactions and congenital analbuminemia.

Test principle

Immunoturbidimetric assay.
Anti-albumin antibodies react with the antigen in the sample to form antigen/antibody complexes which, following agglutination, are measured turbidimetrically.

Reagents - working solutions

R1 TRIS buffer: 50 mmol/L, pH 8.0; PEG: 4.2 %; EDTA: 2.0 mmol/L; preservative

R2 Polyclonal anti-human albumin antibodies (sheep): dependent on titer; TRIS buffer: 100 mmol/L, pH 7.2; preservative

R3 Reagent for antigen excess check.

Albumin in diluted serum (human); NaCl: 150 mmol/L; phosphate buffer: 50 mmol/L, pH 7.0; preservative

R1 is in position A, R2 is in position B and R3 is in position C.

Precautions and warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.

All human material should be considered potentially infectious. All products derived from human blood are prepared exclusively from the blood of donors tested individually and shown to be free from HBsAg and antibodies to HCV and HIV.
The testing methods applied were FDA-approved or cleared in compliance with the European Directive 98/79/EC, Annex II, List A.
However, as no testing method can rule out the potential risk of infection with absolute certainty, the material should be handled with the same level of care as a patient specimen. In the event of exposure, the directives of the responsible health authorities should be followed.[8,9]

Reagent handling

Ready for use

Storage and stability

ALBT2

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

Diluent NaCl 9 %

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.
Serum

ALBT2

Tina-quant Albumin CSF

Plasma: Li-heparin and K_2-EDTA plasma
Cerebrospinal fluid

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.

CSF

Stability:[10]	up to 3 days	at 2-8 °C
	6 months	at (-15)-(-25) °C
	indefinitely	at (-60)-(-80) °C

Serum, plasma

Stability:[11]	10 weeks	at 15-25 °C
	5 months	at 2-8 °C
	4 months	at (-15)-(-25) °C

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 sample type CSF

cobas c 501 **test definition**

Assay type	2-Point End		
Reaction time / Assay points	10 / 10-34		
Wavelength (sub/main)	700/340 nm		
Reaction direction	Increase		
Units	mg/L		
Reagent pipetting		Diluent (H_2O)	
R1	100 µL	–	
R2	20 µL	–	
R3	6 µL	20 µL	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	6.0 µL	10 µL	110 µL
Decreased	3.0 µL	5 µL	180 µL
Increased	6.0 µL	10 µL	110 µL

cobas c 502 **test definition**

Assay type	2-Point End		
Reaction time / Assay points	10 / 10-34		
Wavelength (sub/main)	700/340 nm		
Reaction direction	Increase		
Units	mg/L		
Reagent pipetting		Diluent (H_2O)	
R1	100 µL	–	
R2	20 µL	–	
R3	6 µL	20 µL	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	6.0 µL	10 µL	110 µL
Decreased	3 µL	5 µL	180 µL
Increased	12.0 µL	10 µL	110 µL

Application for sample type serum and plasma

cobas c 501 **test definition**

Assay type	2-Point End		
Reaction time / Assay points	10 / 10-34		
Wavelength (sub/main)	700/340 nm		
Reaction direction	Increase		
Units	mg/L		
Reagent pipetting		Diluent (H_2O)	
R1	100 µL	–	
R2	20 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	2.0 µL	2.1 µL	175 µL
Decreased	2.0 µL	1.7 µL	180 µL
Increased	2.0 µL	2.1 µL	175 µL

cobas c 502 **test definition**

Assay type	2-Point End		
Reaction time / Assay points	10 / 10-34		
Wavelength (sub/main)	700/340 nm		
Reaction direction	Increase		
Units	mg/L		
Reagent pipetting		Diluent (H_2O)	
R1	100 µL	–	
R2	20 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	2.0 µL	2.1 µL	175 µL
Decreased	2.0 µL	1.7 µL	180 µL
Increased	4.0 µL	2.1 µL	175 µL

Calibration

Calibrators S1: H_2O

S2-6: C.f.a.s. PUC

Multiply the lot-specific C.f.a.s. PUC calibrator value by the factors below to determine the standard concentrations for the 6-point calibration curve:

S2: 0.0138 S5: 0.467

S3: 0.0228 S6: 1.00

S4: 0.0455

Calibration mode RCM

ALBT2

Tina-quant Albumin CSF

Calibration frequency	Full calibration - after reagent lot change - and as required following quality control procedures

Traceability: This method has been standardized against the certified reference material in human serum of the IRMM (Institute for Reference Materials and Measurements) ERM-DA470k/IFCC.

Quality control

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

In addition, other suitable control material can be used.

CSF	undiluted Precipath PUC
Serum, plasma	PreciControl ClinChem Multi 1 PreciControl ClinChem Multi 2

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 CSF sample.

To calculate serum/plasma samples in g/L a calculated test must be programmed under Utility > Calculated Test on the Roche/Hitachi **cobas c** 501 analyzer. Please use the following settings.

cobas c 501

Sample Type	Ser/Pl
Unit of Measure	g/L
Report Name	ALBT Serum
Item	ALBTS
Formula	Alb-C/1000

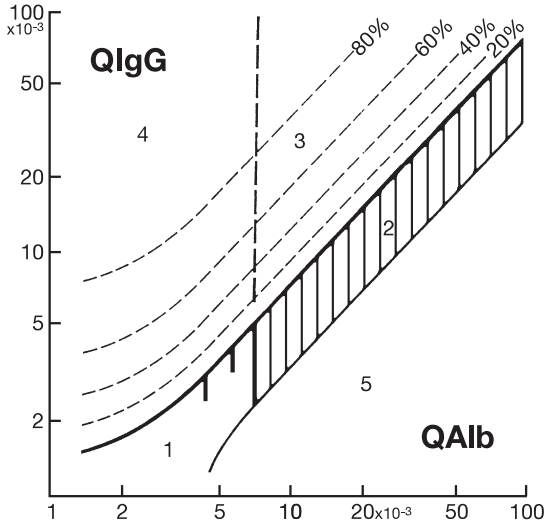
The values for serum/plasma in g/L will be automatically calculated after result output. It is recommended to report the IgG values in serum/plasma to two decimal places, which can be entered in the editable field "Expected Values".

For the definition of the calculated test on the **cobas c** 502 analyzer, refer to the operator's manual of the **cobas** 8000 Data Manager.

Reiber Quotient Graph

With the aid of commercially available software, Reiber Quotient Diagrams can be automatically generated.

The calculation employs a ratio diagram including hyperbolic functions as differential lines according to Reiber and Felgenhauer. Results from the determination of IgG and albumin in CSF and serum (IgG and albumin ratios)[12] are plotted.



1. Reference range. 2. Blood brain barrier functional disorder without local IgG synthesis. 3. Blood brain barrier functional disorder with concomitant IgG-synthesis in the CNS. 4. IgG synthesis in the CNS without blood brain barrier functional disorder. 5. As confirmed empirically, there are no values in this region (i.e. values here are due to errors introduced by blood sampling or analytical errors). Generally speaking, cases not associated with local IgG synthesis in the CNS lie below the bold line (hyperbolic function). The percentage values indicate what percentage of the total IgG in CSF (minimum) originates in the CNS relative to the statistically-defined 0 % differential lines.

Limitations - interference

Serum/plasma

Criterion: Recovery within ± 10 % of initial value at an albumin concentration of 35 g/L.

Icterus:[13] 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:[13] No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 621 µmol/L or 1000 mg/dL).

Lipemia (Intralipid):[13] No significant interference up to an L index of 1500. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Rheumatoid factors ≤ 1200 IU/mL do not interfere.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[14,15]

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[16]

CSF

Criterion: Recovery within ± 10 % of initial value at an albumin concentration of 240 mg/L.

Hemolysis: No significant interference up to a hemoglobin concentration of 620 µmol/L or 1000 mg/dL.

High dose hook-effect: Using the prozone check, no false result without a flag was observed up to an albumin concentration of 30000 mg/L.

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 not required.

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

ALBT2

Tina-quant Albumin CSF

cobas®

Limits and ranges

Measuring range

CSF

36-4800 mg/L

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:6.2 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 6.2.

Serum, plasma

3-101 g/L

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:1.27 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 1.27.

Lower limits of measurement

Limit of Blank and Limit of Detection

CSF

Limit of Blank	= 20 mg/L
Limit of Detection	= 36 mg/L

Serum, plasma

Limit of Blank	= 1 g/L
Limit of Detection	= 2 g/L

The Limit of Blank and Limit of Detection were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A 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 %).

Values below the Limit of Detection (≤ 3 g/L (serum, plasma); ≤ 36 mg/L (CSF)) will not be flagged by the instrument.

Expected values

Serum/plasma

Reference Range Study:[17]

Adults	35.6-46.1 g/L

Consensus values:[18]

Adults	35-52 g/L

Reference intervals according to Tietz:[19]

Newborns 0-4 days:	28-44 g/L
Children 4 days-14 years:	38-54 g/L
Children 14-18 years:	32-45 g/L

Albumin CSF/serum ratio (Q_{ALB} x 10^3)

Adults:[3]	up to 15 years	5.0
	up to 40 years	6.5
	up to 60 years	8.0

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:

CSF

Repeatability	*Mean*	*SD*	*CV*
	mg/L	*mg/L*	*%*
Precipath PUC	99.2	1.4	1.4
Human CSF 1	174	3	1.7
Human CSF 2	383	4	1.0
C.f.a.s. PUC	454	4	0.8

Intermediate precision	*Mean*	*SD*	*CV*
	mg/L	*mg/L*	*%*
Precipath PUC	91.0	2.9	3.2
Human CSF 1	389	7	1.7
Human CSF 3	166	4	2.3
Human CSF 4	366	5	1.3

Serum/plasma

Repeatability	*Mean*	*SD*	*CV*
	g/L	*g/L*	*%*
Precinorm Protein	39.9	0.5	1.2
Precipath Protein	66.6	1.4	2.1
Human serum 1	27.6	0.3	1.3
Human serum 2	62.5	0.9	1.5

Intermediate precision	*Mean*	*SD*	*CV*
	g/L	*g/L*	*%*
Precinorm Protein	42.3	0.9	2.0
Precipath Protein	70.5	1.6	2.2
Human serum 3	7.78	0.74	9.5
Human serum 4	36.2	0.7	2.1

Method comparison

Serum/plasma

Albumin values for human serum and plasma samples obtained on a Roche/Hitachi **cobas c** 501 analyzer (y) were compared with those determined with a nephelometric albumin test (x).

Sample size (n) = 80

Passing/Bablok[20]	Linear regression
y = 0.950x + 0.195 g/L	y = 0.941x + 0.581 g/L
τ = 0.923	r = 0.993

The sample concentrations were between 5.70 and 107 g/L.

CSF

Albumin values for human CSF samples obtained on a Roche/Hitachi **cobas c** 501 analyzer (y) were compared with those determined with a nephelometric albumin test (x).

Sample size (n) = 85

Passing/Bablok[20]	Linear regression
y = 1.00x - 8.75 mg/L	y = 0.991x + 0.301 mg/L
τ = 0.936	r = 0.992

The sample concentrations were between 115 and 2640 mg/L.

ALBT2

Tina-quant Albumin CSF

cobas®

References

1 Greiling H, Gressner AM, eds. Lehrbuch der Klinischen Chemie und Pathobiochemie, 3rd ed. Stuttgart/New York: Schattauer Verlag 1995:749-750.

2 Rothschild MA, Oratz M, Schreiber SS. Serum albumin. Hepatology 1988;8(2):385-401.

3 Reiber H. External Quality Assessment in Clinical Neurochemistry: Survey of Analysis for Cerebrospinal Fluid (CSF) Proteins based on CSF/Serum Quotients. Clin Chem 1995;41:256-263.

4 Reiber H, Felgenhauer K. Protein transfer of the blood cerebrospinal fluid barrier and the quantitation of the humoral immune response within the central nervous system. Clin Chim Acta 1987;163(3):319-328.

5 Reiber H. Die diagnostische Bedeutung neuroimmunologischer Reaktionsmuster im Liquor cerebrospinalis. Lab med 1995;19:444-462.

6 Zimmermann K, Marr U, Linke E. Liquordiagnostik, MTA 1996;11:258-260.

7 Reiber H. Flow rate of cerebrospinal fluid (CSF) – a concept common to normal blood-CSF barrier function and to dysfunction in neurological diseases. J Neurol Sci 1994;122:189-203.

8 Occupational Safety and Health Standards: bloodborne pathogens. (29 CFR Part 1910.1030). Fed. Register.

9 Directive 2000/54/EC of the European Parliament and Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work.

10 Tietz NW, ed. Clinical Guide to Laboratory Tests, 3rd ed. Philadelphia, PA: WB Saunders Company 1995;24.

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

12 Reiber H. The hyperbolic function: a mathematical solution of the protein flux/CSF flow model for blood-CSF barrier function. J Neurol Sci 1994;126:243-245.

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

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

15 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.

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

17 Junge W, Bossert-Reuther S, Klein G, et al. Reference Range Study for Serum Albumin using different methods. Clin Chem Lab Med (June 2007 Poster EUROMEDLAB) 2007;45 Suppl:194.

18 Dati F, Schumann G, Thomas L, et al. Consensus of a group of professional societies and diagnostic companies on guidelines for interim reference ranges for 14 proteins in serum based on the standardization against the IFCC/BCR/CAP reference material (CRM 470). Eur J Clin Chem Clin Biochem 1996;34:517-520.

19 Burtis CA, Ashwood ER, Bruns DE (eds.). Tietz Textbook of Clinical Chemistry and Molecular Diagnostics. 4th ed. St Louis, Missouri; Elsevier Saunders 2006;549.

20 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.

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 after reconstitution or mixing
GTIN	Global Trade Item Number

CE

Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
www.roche.com

Roche