IGG-2

cobas®

Tina-quant IgG CSF

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
03507432 190	Tina-quant IgG Gen.2 (150 tests)	System-ID 07 6787 5	Roche/Hitachi **cobas c** 501/502
03121305 122	Calibrator f.a.s. PUC (5 × 1 mL)	Code 489	
03121313 122	Precinorm PUC (4 × 3 mL)	Code 240	
03121291 122	Precipath PUC (4 × 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:

IGG-C: ACN 119

For **cobas c** 502 analyzer:

IGG-C: ACN 8119

Intended use

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

Summary[1,2,3]

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 in CSF are often associated with opportunistic infections of the central nervous system (CNS) and neurotuberculosis.
Increased CSF IgG concentrations may occur because of either increased permeability of the blood-brain barrier or local/intrathecal production of IgG, 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 and albumin in CSF/serum pairs, a differentiation between IgG originating from blood and IgG originating from intrathecal production is possible. The results of the CSF/serum ratio for IgG and Albumin, in conjunction with Reiber quotient scheme provide an aid in the diagnosis of functional blood-brain barriers disorders and/or intrathecal IgG synthesis.
IgG molecules are composed of two light chains (kappa or lambda) and two gamma heavy chains. Approximately 80 % of serum immunoglobulin is IgG; its main tasks are the defense against microorganisms, direct neutralization of toxins and induction of complement fixation.

Test principle

Immunoturbidimetric assay.

Anti-IgG antibodies react with antigen in the sample to form an antigen/antibody complex. Following agglutination, this is measured turbidimetrically. Addition of PEG allows the reaction to progress rapidly to the end point, increases sensitivity, and reduces the risk of samples containing excess antigen producing false negative results.

Reagents - working solutions

R1 TRIS buffer: 20 mmol/L, pH 8.0; NaCl: 200 mmol/L; polyethylene glycol: 3.6 %; preservative; stabilizers

R2 Anti-human IgG antibody (goat): dependent on titer; TRIS buffer: 20 mmol/L, pH 8.0; NaCl: 150 mmol/L; preservative

R1 is in position B and R2 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.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Danger

H318	Causes serious eye damage.

Prevention:

P280	Wear eye protection/ face protection.

Response:

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 or doctor/ physician.

Product safety labeling primarily follows EU GHS guidance.

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

Reagent handling

Ready for use

Storage and stability

IGG-2

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

Pairs of CSF/serum or CSF/plasma should be collected at the same time.

For specimen collection and preparation only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable.

IGG-2

Tina-quant IgG CSF

Serum

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.

Serum and plasma

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

CSF

Samples should be as fresh as possible. Centrifuge samples containing particles and/or cells before performing the assay.

Stability:[4]	1 day at 15-25 °C
	7 days at 2-8 °C
	Storage at (-15)-(-25) °C is not recommended.

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 / 10-46		
Wavelength (sub/main)	700/340 nm		
Reaction direction	Increase		
Units	mg/L		
Reagent pipetting		Diluent (H_2O)	
R1	120 µL	–	
R2	10 µL	20 µL	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	14.5 µL	–	–
Decreased	2.9 µL	–	–
Increased	14.5 µL	–	–

cobas c 502 **test definition**

Assay type	2-Point End		
Reaction time / Assay points	10 / 10-46		
Wavelength (sub/main)	700/340 nm		
Reaction direction	Increase		
Units	mg/L		
Reagent pipetting		Diluent (H_2O)	
R1	120 µL	–	
R2	10 µL	20 µL	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	14.5 µL	–	–
Decreased	2.9 µL	–	–
Increased	29 µL	–	–

Application for sample type serum and plasma

cobas c 501 **test definition**

Assay type	2-Point End		
Reaction time / Assay points	10 / 10-46		
Wavelength (sub/main)	700/340 nm		
Reaction direction	Increase		
Units	mg/L		
Reagent pipetting		Diluent (H_2O)	
R1	120 µL	–	
R2	10 µL	20 µL	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	2.9 µL	3 µL	147 µL
Decreased	2.9 µL	3 µL	147 µL
Increased	2.9 µL	3 µL	147 µL

cobas c 502 **test definition**

Assay type	2-Point End		
Reaction time / Assay points	10 / 10-46		
Wavelength (sub/main)	700/340 nm		
Reaction direction	Increase		
Units	mg/L		
Reagent pipetting		Diluent (H_2O)	
R1	120 µL	–	
R2	10 µL	20 µL	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	2.9 µL	3 µL	147 µL
Decreased	2.9 µL	3 µL	147 µL
Increased	5.8 µL	3 µL	147 µL

Calibration

Calibrators	S1: H_2O
	S2-S6: C.f.a.s. PUC
	Multiply the lot-specific C.f.a.s. PUC calibrator value by the factors below to determine the standard calibration curve:
	S2: 0.0431 S5: 0.331
	S3: 0.0862 S6: 1.00
	S4: 0.166
Calibration mode	RCM

IGG-2

Tina-quant IgG CSF

cobas®

Calibration frequency	Full calibration
	- after reagent lot change
	- 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.[5]

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	Precinorm PUC
	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	IgG Serum
Item	IGGS
Formula	IGG-C/1000

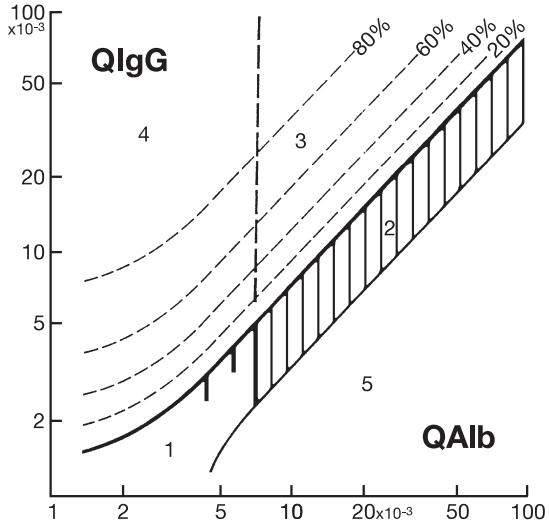
The values for serum/plasma in g/L will be automatically calculated after result output. It is recommended to report the IgG values in serum/plasma to two decimal places, which can be entered in the editable field "Expected Values".

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)[6] 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 IgG concentration of 7.00 g/L.

Icterus:[7] 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:[7] No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 621 µmol/L or 1000 mg/dL).

Lipemia (Intralipid):[7] No significant interference up to an L index of 2000 (approximate intralipid concentration: 2000 mg/dL). There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Rheumatoid factors < 1200 IU/mL do not interfere.

High dose hook-effect: No false result without a flag up to an IgG concentration of 400 g/L occurs due to an antigen excess within polyclonal specimens.

There is no cross-reaction between IgG and IgA or IgM under the assay conditions.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[8,9]

As with other turbidimetric or nephelometric procedures, this test may not provide accurate results in patients with monoclonal gammopathy, due to individual sample characteristics which can be assessed by electrophoresis.[10]

The assay was designed for the determination of IgG in serum/CSF or plasma/CSF pairs only. This assay shall not be used to determine IgG in serum or plasma alone, but always in combination with the matching CSF samples.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

CSF

Criterion: Recovery within ± 10 % of initial value at an IgG concentration of 15.00 mg/L.

Icterus: No significant interference up to a conjugated and unconjugated bilirubin concentration of 257 µmol/L (15 mg/dL).

Hemolysis: No significant interference up to a hemoglobin concentration of 124 µmol/L (200 mg/dL).

High dose hook-effect: No false result without a flag up to an IgG concentration of 2500 mg/L occurs due to an antigen excess within polyclonal specimens.

There is no cross-reaction between IgG and IgA or IgM under the assay conditions.

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.

Limits and ranges

Measuring range

CSF

4.00-200 mg/L

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.

Serum/plasma

3.00-50.0 g/L

Lower limits of measurement

Lower detection limit of the test

CSF

4.00 mg/L

Serum/plasma

0.30 g/L

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 the lowest standard (standard 1 + 3 SD, repeatability, n = 21).

Expected values

CSF[11]

10-30 mg/L

These values are only for orientation. The only relevant values are the CSF/serum ratios.

Serum/plasma

Adults[12]	7-16 g/L
Children and juveniles[13]	
0-1 year	2.32-14.11 g/L
1-3 years	4.53-9.16 g/L
4-6 years	5.04-14.65 g/L
7-9 years	5.72-14.74 g/L
10-11 years	6.98-15.60 g/L
12-13 years	7.59-15.50 g/L
14-15 years	7.16-17.11 g/L
16-19 years	5.49-15.84 g/L

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

CSF

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, one lot of reagent, 21 days).

Repeatability	*Mean*	*SD*	*CV*
	mg/L	mg/L	%
Precinorm PUC	18.8	0.3	1.6
Precipath PUC	150	2	1.1
CSF 1	7.62	0.25	3.3
CSF 2	95.5	0.5	0.5
Intermediate precision	*Mean*	*SD*	*CV*
	mg/L	mg/L	%
Precinorm PUC	20.1	0.5	2.5
Precipath PUC	160	2	1.0
CSF 3	21.9	0.5	2.1
CSF 4	137	1	1.1

Serum/plasma

Repeatability and intermediate precision were determined using human samples and controls in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP5 requirements (2 aliquots per run, 2 runs per day, 21 days). The following results were obtained:

Repeatability	*Mean*	*SD*	*CV*
	g/L	*g/L*	%
PreciControl CC[a)] Multi 1	8.07	0.14	1.7
PreciControl CC Multi 2	12.4	0.3	2.1
Human Serum 1	9.58	0.22	2.3
Human Serum 2	7.48	0.18	2.4
Human Serum 3	4.01	0.16	3.9
Human Serum 4	16.0	0.3	1.9
Human Serum 5	24.7	0.5	2.1
Human Serum 6	40.0	1.0	2.5

a) CC = ClinChem

Intermediate precision	*Mean*	*SD*	*CV*
	g/L	g/L	%
PreciControl CC Multi 1	8.07	0.17	2.2
PreciControl CC Multi 2	12.4	0.3	2.3
Human Serum 1	9.58	0.23	2.4
Human Serum 2	7.48	0.18	2.4
Human Serum 3	4.01	0.18	4.4
Human Serum 4	16.0	0.4	2.2
Human Serum 5	24.7	0.6	2.4
Human Serum 6	40.0	1.1	2.8

Method comparison

CSF

IgG values for human CSF samples obtained on a Roche/Hitachi **cobas c** 501 analyzer (y) were compared with those determined using the same reagent on a Roche/Hitachi 917 analyzer (x).

Sample size (n) = 77

Passing/Bablok[14]	Linear regression
y = 1.007x - 2.17 mg/L	y = 0.997x - 1.70 mg/L

τ = 0.941 r = 1.000

The sample concentrations were between 10.7 and 186 mg/L.

Serum/plasma

IgG values for human serum and plasma samples obtained on a Roche/Hitachi **cobas c** 501 analyzer (y) were compared with those determined with IGG-2 Serum/plasma application (x).

Sample size (n) = 139

Passing/Bablok[14]	Linear regression
y = 0.982x + 0.601 g/L	y = 0.952x + 1.018 g/L
τ = 0.974	r = 0.997

The sample concentrations were between 3.12 and 49.8 g/L.

References

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2 Thomas L. Labor und Diagnose. 7. Auflage; TH-Books Verlagsgesellschaft mbH, Frankfurt/Main, 2007;1743-1784.

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

5 Zegers I, Schreiber W, Sheldon J, et al. Certification of proteins in the human serum, ERM-DA470k/IFCC, Report EUR 23431 EN - 2008:1-60 (https://web.jrc.ec.europa.eu/rmcatalogue/detailsrmcatalogue.do?referenceMaterial=DA470k).

6 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.

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

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

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 Attaelmannan M, Levinson SS. Understanding and identifying monoclonal gammopathies. Clin Chem 2000;46(8 Pt 2):1230-1238.

11 Reiber H, Thompson EJ, Grimsley G, et al. Quality Assurance for Cerebrospinal Fluid Protein Analysis: International Consensus by an Internet-based Group Discussion. Clin Chem Lab Med 2003;41:331-337.

12 Konsensuswerte der Deutschen Gesellschaft für Laboratoriumsmedizin, der Deutschen Gesellschaft für Klinische Chemie und des Verbandes der Diagnostica-Industrie e.V. (VDGH). Clin Lab 1995;41:743-748.

13 Soldin JS, Brugnara C, Wong EC. Pediatric Reference Intervals. AACC Press, 5th ed., 2005.

14 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

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