IGM-C

cobas®

Tina-quant IgM CSF

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
05973066 190	Tina-quant IgM CSF 150 tests	System-ID 07 7481 1	Roche/Hitachi **cobas c** 311, **cobas c** 501/502
06533850 190	Calibrator f.a.s. IgA/IgM CSF (3 × 1 mL)	Code 408	
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** 311/501 analyzers:

IGM-C: ACN 484

For **cobas c** 502 analyzer:

IGM-C: ACN 8484

Intended use

In vitro test for the quantitative determination of IgM specifically in human cerebrospinal fluid and corresponding human serum / plasma on Roche/Hitachi **cobas c** 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 IgM in CSF are often associated with opportunistic infections of the central nervous system (CNS), neuroborreliosis and Mumps meningoencephalitis. Increased CSF IgM concentrations may occur because of either increased permeability of the blood-brain barrier or local/intrathecal production of 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 synthetized 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 IgM and albumin in CSF /serum pairs, a differentiation between IgM originating from blood and IgM originating from intrathecal production is possible.
The results of the CSF/serum ratio for IgM and Albumin, in conjunction with Reiber quotient scheme provide an aid in the diagnosis of functional blood-brain barriers disorders and/or intrathecal IgM synthesis.
IgM normally consists of 10 heavy µ-chains and 10 kappa or lambda type light chains which are always identical within a molecule. There is also a J-chain linking all the µ-chains together, and disulfide bonds linking two heavy chains, so that simply speaking, IgM has a pentameric structure when compared to that of IgG. IgM is the largest immunoglobulin molecule (MW = 970000), but makes up only 6 % of the plasma immunoglobulins.

Test principle

Particle-enhanced immunoturbidimetric assay

Human IgM agglutinates with latex particles coated with polyclonal anti-human IgM antibodies. The precipitate is determined turbidimetrically.

Reagents - working solutions

R1 TRIS buffer: 500 mmol/L, pH 8.5; NaCl: 1000 mmol/L; rabbit globulin: 0.10 %; detergents; preservative

R2 Latex particles coated with anti-human IgM antibodies (rabbit) in TRIS buffer: 10 mmol/L, pH 8.3; stabilizer; 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.

Reagent handling

Ready for use

Carefully invert reagent container several times prior to use to ensure that the reagent components are mixed.

Storage and stability

IGM-C

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.
Cerebrospinal fluid (CSF).
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.

Serum and plasma

Stability:[4] 2 months at 15-25 °C
4 months at 2-8 °C
6 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

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Tina-quant IgM CSF

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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 311 **test definition**

Assay type	2-Point End		
Reaction time / Assay points	10 / 7-57		
Wavelength (sub/main)	800/570 nm		
Reaction direction	Increase		
Units	mg/L		
Reagent pipetting		Diluent (H_2O)	
R1	120 µL	–	
R2	40 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	14 µL	–	–
Decreased	14 µL	20 µL	80 µL
Increased	28 µL	–	–

cobas c 501/502 **test definition**

Assay type	2-Point End		
Reaction time / Assay points	10 / 13-70		
Wavelength (sub/main)	800/570 nm		
Reaction direction	Increase		
Units	mg/L		
Reagent pipetting		Diluent (H_2O)	
R1	120 µL	–	
R2	40 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	14 µL	–	–
Decreased	14 µL	20 µL	80 µL
Increased	28 µL	–	–

Application for sample type serum and plasma

cobas c 311 **test definition**

Assay type	2-Point End		
Reaction time / Assay points	10 / 7-57		
Wavelength (sub/main)	800/570 nm		
Reaction direction	Increase		
Units	mg/L		
Reagent pipetting		Diluent (H_2O)	
R1	120 µL	–	
R2	40 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	3.5 µL	2 µL	166 µL
Decreased	2 µL	1.1 µL	180 µL
Increased	2 µL	7 µL	161 µL

cobas c 501/502 **test definition**

Assay type	2-Point End		
Reaction time / Assay points	10 / 13-70		
Wavelength (sub/main)	800/570 nm		
Reaction direction	Increase		
Units	mg/L		
Reagent pipetting		Diluent (H_2O)	
R1	120 µL	–	
R2	40 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	3.5 µL	2 µL	166 µL
Decreased	2 µL	1.1 µL	180 µL
Increased	2 µL	7 µL	161 µL

Calibration

Calibrators	S1: H_2O	
	S2-S6: C.f.a.s. IgA/IgM CSF	
	Multiply the lot-specific C.f.a.s. IgA/IgM CSF calibrator value by the factors below to determine the standard concentrations for the 6-point calibration curve:	
cobas c 311	S2: 0.01506	S5: 0.1505
	S3: 0.04515	S6: 0.2408
	S4: 0.1003	
cobas c 501/502	S2: 0.01563	S5: 0.1563
	S3: 0.04688	S6: 0.2500
	S4: 0.1042	
Calibration mode	RCM2	
Calibration frequency	Full calibration	
	- after reagent lot change	
	- as required following quality control procedures	

Traceability: This method has been standardized against the reference preparation of the IRMM (Institute for Reference Materials and Measurements) ERM-DA 470K.[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:	commercially available control materials
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** 311 analyzer and the Roche/Hitachi **cobas c** 501 analyzer. Please use the following settings.

cobas c 311

Sample Type	Ser/Pl
Unit of Measure	g/L
Report Name	IgM Serum
Item	IGMS
Formula	IGM-C/936.0

cobas c 501

Sample Type	Ser/Pl
Unit of Measure	g/L
Report Name	IgM Serum
Item	IGMS
Formula	IGM-C/1000

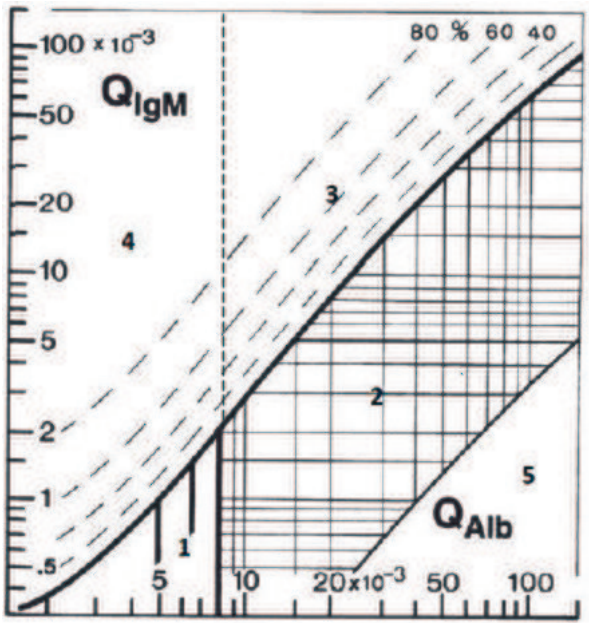
The values for serum/plasma in g/L will be automatically calculated after result output. It is recommended to report the IgM values in serum/plasma to two decimal places, which can be entered in the editable field “Expected Values”.

For the definition of the calculated test on **cobas c** 502, 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 IgM and albumin in CSF and serum (IgM and albumin ratios)[6] are plotted.



1. Reference range. 2. Blood brain barrier functional disorder without local IgM synthesis. 3. Blood brain barrier functional disorder with concomitant IgM-synthesis in the CNS. 4. IgM synthesis in the CNS without blood brain barrier 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 IgM synthesis in the CNS lie below the bold line (hyperbolic function). The percentage values indicate what percentage of the total IgM in CSF (minimum) originates from IgM synthesis in the CNS relative to the statistically-defined 0 % differential lines (bold).

Limitations - interference

CSF:

Criterion: Recovery within ± 10 % of initial value.

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

Hemolysis: No significant interference up to a hemoglobin concentration of 3.11 µmol/L or 5 mg/dL.

High dose hook-effect: Using the prozone check, no false result without a flag was observed up to an IgM concentration of 3000 mg/L.

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

Serum/plasma:

Criterion: Recovery within ± 10 % of initial value.

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. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

High dose hook-effect: Using the prozone check, no false result without a flag was observed up to an IgM concentration of 100 g/L.

There is no cross-reaction between IgM and IgG or IgA 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 IgM in serum/CSF or plasma/CSF pairs only. This assay shall not be used to determine IgM 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.

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/Multiclean/SCCS or 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

0.1-15 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.

Determine samples having lower concentrations via the rerun function. For samples with lower concentrations, the rerun function increases the sample volume by a factor of 2. The results are automatically divided by this factor.

Serum/plasma:

0.1-5 g/L

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:3.7 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 3.7.

Determine samples having lower concentrations via the rerun function. For samples with lower concentrations, the rerun function increases the sample volume by a factor of 2. The results are automatically divided by this factor.

Lower limits of measurement

Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ)

CSF:

Limit of Blank	= 0.05 mg/L
Limit of Detection	= 0.1 mg/L
Limit of Quantitation	= 0.25 mg/L

Serum/plasma:

Limit of Blank	= 0.05 g/L
Limit of Detection	= 0.1 g/L
Limit of Quantitation	= 0.25 g/L

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 %).

Values below the Limit of Detection (< 0.1 mg/L for CSF and < 0.1 g/L for serum/plasma) will not be flagged by the instrument.

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 IgM samples.

Expected values

CSF[11]

0.5-1.5 mg/L

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

Serum/plasma

Reference values according to CRM 470 Protein Standardization:[12,13]

Adults	0.4-2.3 g/L
Children and juveniles	
0-1 year	0.00-1.45 g/L
1-3 years	0.19-1.46 g/L
4-6 years	0.24-2.10 g/L
7-9 years	0.31-2.08 g/L
10-11 years	0.31-1.79 g/L
12-13 years	0.35-2.39 g/L
14-15 years	0.15-1.88 g/L
16-19 years	0.23-2.59 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

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:

CSF:

Repeatability	*Mean mg/L*	*SD mg/L*	*CV %*
Control 1	2.58	0.02	0.9
Control 2	7.30	0.09	1.2
CSF 1	0.311	0.014	4.7
CSF 2	1.28	0.02	1.7
CSF 3	7.90	0.13	1.7
CSF 4	13.5	0.4	3.1

Intermediate precision	*Mean mg/L*	*SD mg/L*	*CV %*
Control 1	2.58	0.03	1.0
Control 2	7.30	0.11	1.5
CSF 1	0.311	0.016	5.2
CSF 2	1.28	0.04	2.9
CSF 3	7.90	0.22	2.8
CSF 4	13.5	0.4	3.1

Serum/plasma:

Repeatability	*Mean g/L*	*SD g/L*	*CV %*
PreciControl CC Multi 1	0.635	0.009	1.3
PreciControl CC Multi 2	0.915	0.011	1.2
Human serum 1	0.327	0.006	1.8
Human serum 2	2.30	0.04	1.6
Human serum 3	2.81	0.04	1.5
Human serum 4	4.57	0.09	2.0

Intermediate precision	*Mean g/L*	*SD g/L*	*CV %*
PreciControl CC Multi 1	0.635	0.009	1.4
PreciControl CC Multi 2	0.915	0.015	1.6
Human serum 1	0.327	0.007	2.1
Human serum 2	2.30	0.04	1.9
Human serum 3	2.81	0.05	1.7
Human serum 4	4.57	0.11	2.3

Method comparison

CSF:

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

Sample size (n) = 281

Passing/Bablok[14]	Linear regression
y = 0.930x + 0.0107 mg/L	y = 0.828x + 0.108 mg/L
τ = 0.923	r = 0.987

The sample concentrations were between 0.142 and 17.5 mg/L.

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Tina-quant IgM CSF

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Serum/plasma:

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

Sample size (n) = 356

Passing/Bablok[14]	Linear regression
y = 0.906x - 0.0182 g/L	y = 0.853x + 0.0332 g/L
τ = 0.934	r = 0.992

The sample concentrations were between 0.209 and 6.39 g/L.

References

1 Reiber H, Peter JB. Cerebrospinal fluid analysis: disease-related data patterns and evaluation programs. J Neurol Sci 2001;184:101-122.

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

3 Davis AC, Shulman MJ. IgM – Molecular requirements for its assembly and function. Immunology Today 1989;10(4):118-128.

4 WHO Publication: Use of anticoagulants in diagnostic laboratory investigations, 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).

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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.

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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.

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13 Lockitch G, Halstead AC, Quigley G, et al. Age- and sex-specific pediatric reference intervals; study design and methods illustrated by measurement of serum proteins with the Behring LN Nephelometer. Clin Chem 1988;34:1618-1621.

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

CE

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

Roche