IGG-2

cobas®

Tina-quant IgG Gen.2 CSF

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
08057915190	Tina-quant IgG Gen.2 (300 tests)	System-ID 2074 001	**cobas c** 303, **cobas c** 503, **cobas c** 703

Materials required (but not provided):

03121305122	Calibrator f.a.s. PUC (5 × 1 mL)	Code 20489	
03121313122	Precinorm PUC (4 × 3 mL)	Code 20240	
03121291122	Precipath PUC (4 × 3 mL)	Code 20241	
05117003190	PreciControl ClinChem Multi 1 (20 × 5 mL)	Code 20391	
05947626190	PreciControl ClinChem Multi 1 (4 × 5 mL)	Code 20391	
05117216190	PreciControl ClinChem Multi 2 (20 × 5 mL)	Code 20392	
05947774190	PreciControl ClinChem Multi 2 (4 × 5 mL)	Code 20392	
08063494190	Diluent NaCl 9 % (123 mL)	System-ID 2906 001	

English

System information

IGG2C: ACN 20741 (sensitive application for cerebrospinal fluid)

IGG2-SR: ACN 20744 (application in serum/plasma)

Intended use

In vitro test for the quantitative determination of IgG specifically in human cerebrospinal fluid and corresponding human serum/plasma on **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 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. By 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 2 light chains (kappa or lambda) and 2 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

R3 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 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:

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/ doctor.

Product safety labeling follows EU GHS guidance.

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

Reagent handling

Ready for use

Storage and stability

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	26 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.
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.

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

Stability in serum/plasma:[4]	4 months at 15-25 °C
	8 months at 2-8 °C
	8 months at -20 °C (±5 °C)
Stability in CSF:[4]	1 day at 15-25 °C
	7 days at 2-8 °C
	Storage at -20 °C (±5 °C) is not recommended.

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

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

Test definition

Reporting time	10 min		
Wavelength (sub/main)	700/340 nm		
Reagent pipetting		Diluent (H_2O)	
R1	95 µL	–	
R3	8 µL	16 µL	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	11.5 µL	–	–
Decreased	11.5 µL	20 µL	80 µL
Increased	11.5 µL	–	–

Application for sample type serum and plasma

Test definition

Reporting time	10 min		
Wavelength (sub/main)	700/340 nm		
Reagent pipetting		Diluent (H_2O)	
R1	95 µL	–	
R3	8 µL	16 µL	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	2.3 µL	2 µL	98 µL
Decreased	2.3 µL	2 µL	98 µL
Increased	2.3 µL	2 µL	98 µL

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

Calibration

Application for CSF (ACN 20741)

Calibrators	S1: H_2O
	S2-S6: C.f.a.s. PUC
Calibration mode	Non-linear
Calibration frequency	Automatic full calibration - after reagent lot change
	Full calibration - as required following quality control procedures

Application for serum/plasma (ACN 20744)
Transfer of calibration from CSF application (ACN 20741)

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

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. 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

CSF

cobas c systems automatically calculate the analyte concentration of each sample in the unit mg/L (nmol/L, g/L, mg/dL).

Conversion factors:	mg/L × 6.67 = nmol/L
	mg/L × 0.001 = g/L
	mg/L × 0.1 = mg/dL

Serum/plasma

cobas c systems automatically calculate the analyte concentration of each sample in the unit mg/L (g/L, mg/dL).

Conversion factors:	mg/L × 0.001 = g/L
	mg/L × 0.1 = mg/dL
	g/L × 6.67 = µmol/L

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.

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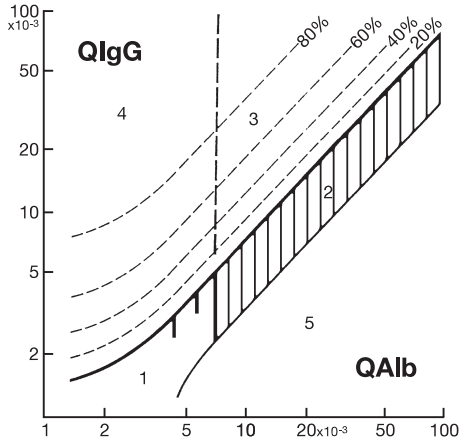

1. Reference range. 2. Blood brain barrier functional disorder without local IgG synthesis. 3. Blood brain barrier functional disorder with concomitant IgG synthesis in the CNS. 4. IgG synthesis in the CNS without blood brain barrier functional disorder. 5. As confirmed empirically, there are no values 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 7.0 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: No significant interference from rheumatoid factors up to a concentration of 1200 IU/mL.

High dose hook-effect: No false result without a flag was observed up to an IgG concentration of 400 g/L.

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 mg/L.

Icterus: No significant interference up to an I index of 15 for conjugated bilirubin (approximate conjugated bilirubin concentration: 257 µmol/L or 15 mg/dL).

Hemolysis: No significant interference up to an H index of 200 (approximate hemoglobin concentration: 124 µmol/L or 200 mg/dL).

High dose hook-effect: No false result without a flag was observed up to an IgG concentration of 2500 mg/L.

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 **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

CSF

4.00-200 mg/L (26.7-1334 nmol/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

3.0-50 g/L (20.0-334 µmol/L)

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

CSF:

Limit of Blank	= 3 mg/L (20.0 nmol/L)
Limit of Detection	= 4 mg/L (26.7 nmol/L)
Limit of Quantitation	= 7 mg/L (46.7 nmol/L)

Serum/plasma:

Limit of Blank	= 0.3 g/L (2.0 µmol/L)
Limit of Detection	= 0.5 g/L (3.34 µmol/L)
Limit of Quantitation	= 0.7 g/L (4.67 µmol/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 %).

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 IgG samples.

Expected values

CSF[11]

10-30 mg/L (66.7-200 nmol/L*)

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

Serum/plasma

Adults:[12] 7-16 g/L (46.7-107 µmol/L*)

Children/juveniles:[13]
0 - 14 days: 3.20-12.1 g/L (21.3-80.4 µmol/L*)
15 days - <1 yr: 1.48-6.31 g/L (9.87-42.1 µmol/L*)
1 - <4 yr: 3.17-9.94 g/L (21.1-66.3 µmol/L*)
4 - <10 yr: 5.01-11.7 g/L (33.4-77.7 µmol/L*)
10 - <19 yr: 5.95-13.1 g/L (39.7-87.2 µmol/L*)

*calculated by unit conversion factor

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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.

CSF:

Repeatability	*Mean mg/L*	*SD mg/L*	*CV %*
Precinorm PUC	23.6	0.351	1.5
Precipath PUC	139	0.715	0.5
CSF 1	9.91	0.224	2.3
CSF 2	25.9	0.317	1.2
CSF 3	38.0	0.280	0.7
CSF 4	104	0.824	0.8
CSF 5	168	1.16	0.7

Intermediate precision	*Mean mg/L*	*SD mg/L*	*CV %*
Precinorm PUC	23.6	0.488	2.1
Precipath PUC	139	1.30	0.9
CSF 1	9.91	0.449	4.5
CSF 2	25.9	0.550	2.1
CSF 3	38.0	0.572	1.5
CSF 4	104	1.04	1.0
CSF 5	168	1.85	1.1

Serum/plasma:

Repeatability	*Mean g/L*	*SD g/L*	*CV %*
PCCC1[a)]	6.96	0.0906	1.3
PCCC2[b)]	10.7	0.0794	0.7
Human serum 1	3.40	0.0553	1.6
Human serum 2	6.93	0.0529	0.8
Human serum 3	16.0	0.105	0.7
Human serum 4	24.0	0.159	0.7
Human serum 5	42.5	0.352	0.8

Intermediate precision	*Mean g/L*	*SD g/L*	*CV %*
PCCC1[a)]	6.96	0.115	1.6
PCCC2[b)]	10.7	0.153	1.4
Human serum 1	3.40	0.0909	2.7
Human serum 2	6.66	0.112	1.7
Human serum 3	15.7	0.203	1.3
Human serum 4	24.0	0.299	1.2
Human serum 5	42.5	0.676	1.6

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) and **cobas c** 703 analyzer(s).

Method comparison

IgG values for human CSF, serum and plasma 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).

CSF:

Sample size (n) = 83

Passing/Bablok[14]	Linear regression
y = 1.003x + 1.37 mg/L	y = 0.971x + 2.66 mg/L
τ = 0.978	r = 0.998

The sample concentrations were between 4.19 and 193 mg/L.

Serum/plasma:

Sample size (n) = 84

Passing/Bablok[14]	Linear regression
y = 0.960x + 0.315 g/L	y = 0.943x + 0.525 g/L
τ = 0.976	r = 0.998

The sample concentrations were between 3.56 and 49.2 g/L.

IgG values for human CSF, serum and plasma 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).

CSF:

Sample size (n) = 81

Passing/Bablok[14]	Linear regression
y = 1.022x + 1.67 mg/L	y = 1.005x + 2.18 mg/L
τ = 0.988	r = 1.000

The sample concentrations were between 5.33 and 193 mg/L.

Serum/plasma:

Sample size (n) = 74

Passing/Bablok[14]	Linear regression
y = 0.944x + 0.661 g/L	y = 0.911x + 1.09 g/L
τ = 0.983	r = 0.997

The sample concentrations were between 3.73 and 47.1 g/L.

IgG values for human CSF, serum and plasma samples obtained on a **cobas c** 703 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 503 analyzer (x).

CSF:

Sample size (n) = 80

Passing/Bablok[14]	Linear regression
y = 1.013x + 0.0401 mg/L	y = 1.036x - 0.577 mg/L
τ = 0.971	r = 0.996

The sample concentrations were between 4.73 and 191 mg/L.

Serum/plasma:

Sample size (n) = 83

Passing/Bablok[14]	Linear regression
y = 0.998x - 0.0662 g/L	y = 0.996x + 0.0546 g/L
τ = 0.984	r = 1.000

The sample concentrations were between 3.61 and 48.3 g/L.

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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 navifyportal.roche.com for definition of symbols used):

CONTENT	Contents of kit
→	Volume for reconstitution
GTIN	Global Trade Item Number
Rx only	For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

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

CE 0123

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