IGGT

cobas®

Immunoglobulin G (Turbidimetric)

Specific proteins

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
20766631 322	Immunoglobulin G (Turbidimetric) (100 tests)	System-ID 07 6663 1	COBAS INTEGRA 400 plus COBAS INTEGRA 800
20737267 322	Serum proteins T Standard (5 × 0.5 mL)	System-ID 07 3726 7	
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	
10171743 122	Precinorm U (20 × 5 mL)	System-ID 07 7997 0	
10171735 122	Precinorm U (4 × 5 mL)	System-ID 07 7997 0	
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	
20756350 322	NaCl Diluent 9 % (6 × 22 mL)	System-ID 07 5635 0	
20721867 322	Accelerator I (1 × 100 mL)	System-ID 07 2186 7	

English

System information

Test IGGT, test ID 0-276 (serum, plasma)
Test IGGTC, test ID 0-476 (cerebrospinal fluid)

Intended use

In vitro test for the quantitative immunological determination of human immunoglobulin G in serum, plasma and cerebrospinal fluid on COBAS INTEGRA systems.

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

Immunoglobulins protect the human body against invading organisms and agents. Immunoglobulins contain an antigen binding part (Fab portion) and a Fc portion of which the latter can interact with cells of the immune system and the complement factors. The immunoglobulin Fab part recognizes antigens in solution (e.g. toxins) and antigens associated with microorganisms (e.g. bacteria, viruses). The antigen binding site may initiate the direct neutralization of toxins, the sensitization of immunocompetent cells, the reduction of viral infectivity, or the development of an inflammatory reaction.

As a normal result of infections all immunoglobulin classes increase in serum. In addition, raised IgG levels are found during autoimmune diseases and chronic hepatitis. Malignant cell proliferation of an immunoglobulin producing cell (plasma cell) causes a serum level increase of a single immunoglobulin (plasmocytoma). Immunoglobulin deficiencies may be due to protein loss syndromes, inherited deficiencies or may be secondary to lymphoid malignancies.

Infants show a decrease of IgG between 3 and 6 months because maternal IgG is at first only partly compensated by active IgG synthesis of the newborn.

IgG determination in cerebrospinal fluid (CSF) is used for evaluation of infections involving the central nervous system, neoplasms, or primary neurologic diseases (in particular, multiple sclerosis). Increased CSF IgG concentration may occur either because of increased permeability of the blood-brain barrier, or increased local production of IgG, or both. In order to identify intrathecal production specifically, correction for increased permeability is necessary. To accurately determine the intrathecal IgG production, the IgG fraction caused by increased permeability can be corrected by making calculations as follows:[4]

Abbreviated ratio name: IGGR2 (0-179)
Ratio = IgG_{CSF} (mg/L) / $Albumin_{CSF}$ (mg/L)
A ratio > 0.27 indicates increased intrathecal IgG synthesis.

Abbreviated ratio name: IGGI2 (0-180)
IgG index = IgG_{CSF} (mg/L) × $Albumin_{Ser}$ (g/L) / IgG_{Ser} (g/L) / $Albumin_{CSF}$ (mg/L)

Index values > 0.7 are considered indicative of increased IgG synthesis. In > 80 % of multiple sclerosis cases, the index exceeds 0.7.

It is known that the so-called paraproteins secreted in monoclonal gammopathies (monoclonal immunoglobulinemia) may differ from the respective immunoglobulins of polyclonal origin by amino acid composition and size. This may impair the binding to the antibody, and hence impair accurate quantitation.

Test principle[6]

Immunoturbidimetric assay
Human IgG forms a precipitate with a specific antiserum which is determined turbidimetrically at 340 nm.

Reagents - working solutions

R1 Anti-IgG T antiserum (rabbit) specific for human IgG in phosphate buffer; preservative

R2 Reagent for antigen excess check
IgG in diluted serum (human); preservative

R1 is in position A and R2 is in position C.

Precautions and warnings

Pay attention to all precautions and warnings listed in Section 1 / Introduction of this Method Manual.

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.[7,8]

Reagent handling

Ready for use

Storage and stability

Shelf life at 2-8 °C	See expiration date on **cobas c** pack label
COBAS INTEGRA 400 plus system	
On-board in use at 10-15 °C	12 weeks
COBAS INTEGRA 800 system	
On-board in use at 8 °C	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
Plasma: Li-heparin or EDTA plasma
CSF: Avoid blood contamination of CSF samples. CSF specimens should be collected with care to avoid blood contamination, since total protein concentration of whole blood is about 1000 times higher than of normal CSF. Centrifuge before analysis.

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.

Samples and controls are automatically prediluted with NaCl solution by the instrument.

Centrifuge samples containing precipitates before performing the assay.

Stability in serum/plasma :[9]	4 months at 15-25 °C 8 months at 2-8 °C 8 months at (-15)-(-25) °C
Stability in CSF :[9]	1 day at 15-25 °C 7 days at 2-8 °C

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

1. *IGGT, IGGTC*
   NaCl Diluent 9 %, Cat. No. 20756350 322, system-ID 07 5635 0 for automatic sample dilution and standard serial dilutions.
   NaCl Diluent 9 % is stable for 4 weeks on-board
   COBAS INTEGRA 400 plus/800 analyzers.
2. *IGGTC*
   Accelerator I, Cat. No. 20721867 122, system ID 07 2186 7 as special diluent (SD)
   Stability on-board in use: 7 days

Both auxiliary reagents are placed in their predefined rack position.

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.

Applications for serum, plasma, and CSF

COBAS INTEGRA 400 plus test defintion

Measuring mode	Absorbance
Abs. calculation mode	Endpoint
Reaction direction	Increase
Reaction start with	Sample
Antigen excess check	Yes (with R2)
Serum and plasma	
Reaction mode	D-R1-S-R2
Wavelength A	340 nm
Calc. first/last	T_0 /33
Typical prozone effect	> 94 g/L (> 9400 mg/dL or > 627 µmol/L)
Predilution factor	21
Unit	g/L
CSF	
Reaction mode	R1-SD/S-R2
Wavelength A/B	340/652 nm
Calc. first/last	T_0 /33
Typical prozone effect	> 405 mg/L (> 40.5 mg/dL or > 2701 nmol/L)
Predilution factor	No
Unit	mg/L

Pipetting parameters

Serum, plasma		Diluent (H_2O)
R1	140 µL	5 µL
Sample	2 µL	20 µL
R2	5 µL	5 µL
Total volume	177 µL	

CSF		Diluent (H_2O)
R1	100 µL	5 µL
Sample	25 µL	
Special diluent (SD)	30 µL	
R2	5 µL	25 µL
Total volume	190 µL	

COBAS INTEGRA 800 test definition

Measuring mode	Absorbance
Abs. calculation mode	Endpoint
Reaction direction	Increase
Reaction start with	Sample
Antigen excess check	Yes (with R2)
Serum and plasma	
Reaction mode	D-R1-S-R2
Wavelength A	340 nm
Calc. first/last	T_0 /44
Typical prozone effect	> 70 g/L (> 7000 mg/dL or > 467 µmol/L)
Predilution factor	21
Unit	g/L
CSF	
Reaction mode	R1-SD/S-R2
Wavelength A/B	340/652 nm
Calc. first/last	T_0 /44
Typical prozone effect	> 405 mg/L (> 40.5 mg/dL or > 2701 nmol/L)
Predilution factor	No
Unit	mg/L

Pipetting parameters

Serum, plasma	Diluent (H_2O)	
R1	140 µL	5 µL
Sample	2 µL	20 µL
R2	5 µL	5 µL
Total volume	177 µL	

CSF	Diluent (H_2O)	
R1	100 µL	5 µL

Sample	25 µL	
Special diluent (SD)	30 µL	
R2	5 µL	25 µL
Total volume	190 µL	

Calibration

Calibrator	Serum proteins T Standard
Calibration dilution ratio	
Serum, plasma	1:6, 1:12, 1:24, 1:48, 1:96 performed automatically by the instrument
CSF	1:150, 1:300, 1:600, 1:1200, 1:2400, performed automatically by the instrument
Calibration mode	Logit/log 5
Calibration replicate	Duplicate recommended
Calibration interval	Each lot and as required following quality control procedures

Enter the assigned lot-specific IgG value of the undiluted calibrator, indicated in the package insert of the Serum proteins T Standard.

Traceability: This method has been standardized against the certified reference material in human serum of the IRMM (Institute for Reference Materials and Measurements) ERM - DA470/IFCC.

Quality control

Quality control *serum, plasma*

Reference range	Precinorm Protein, Precinorm U or PreciControl ClinChem Multi 1
Pathological range	Precipath Protein or PreciControl ClinChem Multi 2
Quality control *CSF*	Quantitative CSF controls are recommended for routine quality control.
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

COBAS INTEGRA analyzers automatically calculate the analyte concentration of each sample. For more details, please refer to Data Analysis in the Online Help (COBAS INTEGRA 400 plus/800 analyzers).

Conversion factors:[10]	g/L × 6.67 = µmol/L
	g/L × 100 = mg/dL
	mg/L × 6.67 = nmol/L
Molecular weight:	150000

Limitations - interference

Criterion: Recovery within ± 10 % of initial value.

Serum, plasma

Hemolysis:[11] No significant interference.

Icterus:[11] No significant interference.

Lipemia (Intralipid):[11] No significant interference.

Rheumatoid factors: No significant interference up to a rheumatoid factors level of 800 IU/mL.

Drugs: Therapeutic drug interference was tested according to the recommendations of the VDGH[a)]. No interferences were found.

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.[12]

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

a) Verband der Diagnostica und Diagnostica Geräte Hersteller. Refer to section 1 / Introduction of this Method Manual for a list of drugs tested and their concentrations.

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 Method Manual, Introduction, Extra Wash Cycles for further instructions.

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

IMPORTANT

The IgG concentration in serum is much higher than in CSF samples. To achieve high sensitivity the CSF application runs with 25 µL undiluted sample. Therefore, this application is prone to sample carryover of IgG.

To avoid sample carryover on COBAS INTEGRA 800 analyzers running CSF samples in batch mode is mandatory. The Extra wash cycle test (EWC-S, 0-989), as discribed in the Method Manual, Introduction, Extra Wash Cycles, prior to CSF batch testing is mandatory.

Limits and ranges

Measuring range

Serum/plasma

3.0-37.2 g/L (300-3720 mg/dL or 20.0-248 µmol/L) (typical test range)

The upper and lower limits of the measuring range depend on the actual calibrator value.

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:4.7 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 4.7.

Determine samples having lower concentrations via the rerun function. For samples with lower concentrations, the rerun function reduces the sample predilution factor to 5.3. The results are automatically multiplied by the reduced predilution factor.

CSF

2.0-70.9 mg/L (0.20-7.09 mg/dL or 13.3-473 nmol/L) (typical test range)

The upper and lower limits of the measuring range depend on the actual calibrator value.

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:10 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 10.

Lower limits of measurement

Serum/plasma

Lower detection limit of the test:
3.0 g/L (300 mg/dL or 20.0 µmol/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 a zero sample (zero sample + 3 SD, repeatability, n = 30).

CSF

Lower detection limit of the test:
2.0 mg/L (0.20 mg/dL or 13.3 nmol/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 a zero sample (zero sample + 3 SD, repeatability, n = 21).

Expected values

Serum/plasma

	g/L	mg/dL	µmol/L
Adults[13]	7-16	700-1600	46.7-106.7
Children and juveniles[14]			
0-1 year	2.32-14.11	232-1411	15.5-94.0
1-3 years	4.53-9.16	453-916	30.2-61.1
4-6 years	5.04-14.65	504-1465	33.6-97.7
7-9 years	5.72-14.74	572-1474	38.2-98.3
10-11 years	6.98-15.60	698-1560	46.6-104
12-13 years	7.59-15.50	759-1550	50.6-103
14-15 years	7.16-17.11	716-1711	47.7-114
16-19 years	5.49-15.84	549-1584	36.6-106

Roche has not evaluated reference ranges in a pediatric population.

CSF

IgG[15]	10-30 mg/L (1.00-3.00 mg/dL or 66.7-200 nmol/L)
IgG index[4]	0.3-0.7
IgG/ALB ratio[4]	< 0.27

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 for serum/plasma (IGGT)

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 and intermediate precision (2 aliquots per run, 2 runs per day, 20 days). The following results were obtained:

	Level 1	Level 2
Mean	10.2 g/L (1020 mg/dL or 68.0 µmol/L)	24.5 g/L (2450 mg/dL or 163 µmol/L)
CV repeatability	0.94 %	1.4 %
CV intermediate precision	2.1 %	1.9 %

Method comparison

IgG values for human serum and plasma samples obtained on a COBAS INTEGRA 700 analyzer with the COBAS INTEGRA Immunoglobulin G (Turbidimetric) reagent (y) were compared with those determined using commercially available reagents for IgG on a COBAS INTEGRA 700 analyzer (x) and an alternative manufacturer's automated system (turbidimetric determination) (x). Samples were measured in duplicate. Sample size (n) represents all replicates.

		COBAS INTEGRA 700 analyzer
Sample size	(n)	600
Correlation coefficient	(r)	0.998
	(r_s)	0.997
Linear regression		y = 0.970x + 0.234 g/L
Passing/Bablok[16]		y = 0.974x + 0.204 g/L
		Alternative system
Sample size	(n)	600
Correlation coefficient	(r)	0.969
	(r_s)	0.963
Linear regression		y = 0.990x - 0.318 g/L
Passing/Bablok[16]		y = 1.029x - 0.689 g/L

The sample concentrations were between 4.76 and 15.9 g/L (480 and 1590 mg/dL or 32.0 and 106 µmol/L).

Specific performance data for cerebrospinal fluid (IGGTC)

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 and intermediate precision (2 aliquots per run, 2 runs per day, 20 days). The following results were obtained:

	Level 1	Level 2
Mean	34.5 mg/L (3.45 mg/dL or 230 nmol/L)	54.1 mg/L (5.41 mg/dL or 361 nmol/L)
CV repeatability	1.2 %	1.8 %
CV intermediate precision	1.7 %	2.2 %

Method comparison

IgG values for human CSF samples obtained on a COBAS INTEGRA 700 analyzer with the COBAS INTEGRA Immunoglobulin G (Turbidimetric) reagent (y) were compared with those determined using commercially available reagents for IgG on an alternative manufacturer's automated system (nephelometric determination) (x).

Sample size	(n)	97
Correlation coefficient	(r)	0.996
	(r_s)	0.991
Linear regression		y = 0.994x - 1.02 mg/L
Passing/Bablok[16]		y = 0.968x + 0.077 mg/L

The sample concentrations were between 2.7 and 459 mg/L (0.27 and 45.9 mg/dL or 18.0 and 3062 nmol/L).

References

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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.

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

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