IGA

Immunoglobulin A

cobas®

Specific proteins

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
20737755 322	Immunoglobulin A (100 tests)	System-ID 07 3775 5	COBAS INTEGRA 400 plus COBAS INTEGRA 800
20737267 322	Serumproteins T Standard (5 x 0.5 mL)	System-ID 07 3726 7	
10557897 122	Precinorm Protein (3 x 1 mL)	System-ID 07 9105 9	
10557897 160	Precinorm Protein (3 x 1 mL, for USA)	System-ID 07 9105 9	
10171743 122	Precinorm U (20 x 5 mL)	System-ID 07 7997 0	
10171735 122	Precinorm U (4 x 5 mL)	System-ID 07 7997 0	
11333127 122	Precipath Protein (3 x 1 mL)	System-ID 07 9106 7	
11333127 160	Precipath Protein (3 x 1 mL, for USA)	System-ID 07 9106 7	
03121291 122	Precipath PUC (4 x 3 mL)	System-ID 07 6757 3	
05117003 190	PreciControl ClinChem Multi 1 (20 x 5 mL)	System-ID 07 7469 3	
05947626 190	PreciControl ClinChem Multi 1 (4 x 5 mL)	System-ID 07 7469 3	
05947626 160	PreciControl ClinChem Multi 1 (4 x 5 mL, for USA)	System-ID 07 7469 3	
05117216 190	PreciControl ClinChem Multi 2 (20 x 5 mL)	System-ID 07 7470 7	
05947774 190	PreciControl ClinChem Multi 2 (4 x 5 mL)	System-ID 07 7470 7	
05947774 160	PreciControl ClinChem Multi 2 (4 x 5 mL, for USA)	System-ID 07 7470 7	
20756350 322	NaCl Diluent 9 % (6 x 22 mL)	System-ID 07 5635 0	
20721867 322	Accelerator I (100 mL)	System-ID 07 2186 7	

English

System information

Test IGA, test ID 0-075 (standard application)

Test IGAP, test ID 0-175 (sensitive application)

Intended use

In vitro test for the quantitative immunological determination of human immunoglobulin A in serum and plasma on COBAS INTEGRA systems. In addition to the standard application (test IGA, test ID 0-075), the sensitive application (test IGAP, test ID 0-175) is designed for the quantitative determination of low IgA concentrations in e.g. pediatric samples.

Summary[1,2]

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. Raised IgA levels are found in skin, gut, respiratory, and renal infections. Malignant cell proliferation of an immunoglobulin producing cell (plasma cell) causes an increased serum level of a single immunoglobulin (plasmocytoma). Immunoglobulin deficiencies may be due to protein loss syndromes, inherited deficiencies or may be secondary to lymphoid malignancies. Due to the slow onset of IgA synthesis, the IgA concentration in serum of infants is lower than in adults.

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[3]

Immunoturbidimetric assay

Human IgA forms a precipitate with a specific antiserum which is determined turbidimetrically at 340 nm.

Reagents - working solutions

R1 Anti-IgA T antiserum (rabbit) specific for human IgA in phosphate buffer; stabilizers

SR Reagent for antigen excess check
IgA in diluted serum (human); stabilizers

R1 is in position B and SR is in position C.

Precautions and warnings

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

For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

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 used assays approved by the FDA 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.[4,5]

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

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:[6] 8 months at 15-25 °C
8 months at 2-8 °C
8 months at (–15)-(–25) °C

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

1. *IGA, IGAP*

   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. *IGAP*

   Accelerator I as special diluent (SD)

   Stability on-board in use: 7 days

Both auxiliary reagents are placed in their predefined rack positions.

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.

Application for serum and plasma

COBAS INTEGRA 400 plus test definition

Measuring mode	Absorbance
Abs. calculation mode	Endpoint
Reaction direction	Increase
Reaction start with	Sample
Antigen excess check	Yes (with SR)
Unit	g/L
Standard application (IGA)	
Reaction mode	D-R1-S-SR
Wavelength A	340 nm
Calc. first/last	T_0/33
Typical prozone effect	> 21.0 g/L (> 131.25 µmol/L or > 2100 mg/dL)
Predilution factor	21
Sensitive application (IGAP)	
Reaction mode	D-R1-SD/S-SR
Wavelength A/B	340/659 nm
Calc. first/last	T_0/33
Typical prozone effect	> 10.6 g/L (> 66.25 µmol/L or > 1060 mg/dL)
Predilution factor	11

Pipetting parameters

Standard application (IGA)		Diluent (H_2O)
R1	90 µL	10 µL
Sample	5 µL	20 µL
SR	5 µL	5 µL
Total volume	135 µL	

Sensitive application (IGAP)		Diluent (H_2O)
R1	90 µL	10 µL
Sample	5 µL	
Special diluent (SD)	20 µL	
SR	5 µL	5 µL
Total volume	135 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 SR)
Unit	g/L
Standard application (IGA)	
Reaction mode	D-R1-S-SR
Wavelength A	340 nm
Calc. first/last	T_0/44
Typical prozone effect	> 21.0 g/L (> 131.25 µmol/L or > 2100 mg/dL)
Predilution factor	21
Sensitive application (IGAP)	
Reaction mode	D-R1-SD/S-SR
Wavelength A/B	340/652 nm
Calc. first/last	T_0/44
Typical prozone effect	> 9 g/L (> 56.25 µmol/L or > 900 mg/dL)
Predilution factor	11

Pipetting parameters

Standard application (IGA)		Diluent (H_2O)
R1	90 µL	10 µL
Sample	5 µL	20 µL
SR	5 µL	5 µL
Total volume	135 µL	

Sensitive application (IGAP)		Diluent (H_2O)
R1	90 µL	10 µL
Sample	5 µL	
Special diluent (SD)	20 µL	
SR	5 µL	5 µL
Total volume	135 µL	

Note

The sensitive application (IGAP) is designed for IgA determination in samples with low IgA concentrations (e.g. pediatric specimens).

Calibration

Calibrator	Serumproteins T Standard
Calibration dilution ratio	
Standard application	1:6, 1:12, 1:24, 1:48, 1:96 performed automatically by the instrument

Sensitive application	1:6, 1:12, 1:24, 1:48, 1:96, 1:192 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

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

Enter the assigned lot-specific IgA value of the undiluted calibrator, indicated in the package insert of the Serumproteins T Standard.

Traceability: This method has been standardized with regard to the IFCC/BCR/CAP reference preparation CRM 470 (RPPHS 91/0619) for 14 serum proteins.[7]

Quality control

Reference range for IGA	Precinorm Protein, Precinorm U or PreciControl ClinChem Multi 1
Pathological range for IGA	Precipath Protein or PreciControl ClinChem Multi 2
Reference range for IGAP	Precipath PUC
Pathological range for IGAP	Precinorm Protein or PreciControl ClinChem Multi 1
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:	g/L × 6.25 = µmol/L
	µmol/L × 0.16 = g/L

Limitations - interference

Criterion: Recovery within ± 10 % of initial value.

Icterus:[8] 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:[8] No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 621 µmol/L or 1000 mg/dL).

Lipemia (Intralipid):[8]
Standard application (IGA): No significant interference up to an L index of 750.
Sensitive application (IGAP): No significant interference.
There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Rheumatoid factors: No significant interference up to a rheumatoid factors level of 500 IU/mL.

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.[9]

Sensitive application (IGAP): For IgA concentrations below 1 g/L (6.25 µmol/L) pathologically high levels of albumin (70 g/L) decrease the apparent IgA concentrations significantly.

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 CLEAN Method Sheet for further instructions and for the latest version of the Extra wash cycle list.
Where required, special wash/carry-over evasion programming must be implemented prior to reporting results with this test.

Limits and ranges

Measuring range

Standard application (IGA)

0.6-7.3 g/L (3.75-45.63 µmol/L or 60-730 mg/dL) (typical measuring range)

The upper limit of the measuring range depends 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.

Determine samples having lower concentrations via the rerun function. For samples with lower concentrations, the rerun function reduces the sample predilution factor to 7. The results are automatically multiplied by the reduced predilution factor.

Sensitive application (IGAP)

0.2-3.54 g/L (1.25-22.13 µmol/L or 20-354 mg/dL) (typical measuring range)

The upper limit of the measuring range depends on the actual calibrator value.

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:3 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 3.

Determine samples having lower concentrations via the rerun function. For samples with lower concentrations, the rerun function reduces the sample predilution factor to 3.7. The results are automatically multiplied by the reduced predilution factor.

Lower limits of measurement

Standard application (IGA)

Lower detection limit of the test:
0.6 g/L (3.75 µmol/L or 60 mg/dL)

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).

Sensitive application (IGAP)

Lower detection limit of the test:
0.2 g/L (1.25 µmol/L or 20 mg/dL)

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).

Expected values

Adults[10]	0.7-4 g/L	(4.38-25.0 µmol/L or 70-400 mg/dL)
Children and juveniles[11]		
0-1 year	0.00-0.83 g/L	(0.00-5.19 µmol/L or 0.00-83 mg/dL)
1-3 years	0.20-1.00 g/L	(1.25-6.25 µmol/L or 20-100 mg/dL)
4-6 years	0.27-1.95 g/L	(1.69-12.19 µmol/L or 27-195 mg/dL)
7-9 years	0.34-3.05 g/L	(2.13-19.06 µmol/L or 34-305 mg/dL)
10-11 years	0.53-2.04 g/L	(3.31-12.75 µmol/L or 53-204 mg/dL)

12-13 years	0.58-3.58 g/L	(3.63-22.38 µmol/L or 58-358 mg/dL)
14-15 years	0.47-2.49 g/L	(2.94-15.56 µmol/L or 47-249 mg/dL)
16-19 years	0.61-3.48 g/L	(3.81-21.75 µmol/L or 61-348 mg/dL)

Roche has not evaluated reference ranges in a pediatric population.

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 COBAS INTEGRA 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:

Standard application (IGA)

	Level 1	Level 2
Mean	2.02 g/L (12.6 µmol/L or 202 mg/dL)	6.15 g/L (38.4 µmol/L or 615 mg/dL)
CV repeatability	2.0 %	0.97 %
CV intermediate precision	2.3 %	1.2 %

Sensitive application (IGAP)

	Level 1	Level 2
Mean	1.17 g/L (7.31 µmol/L or 117 mg/dL)	3.28 g/L (20.5 µmol/L or 328 mg/dL)
CV repeatability	1.1 %	0.96 %
CV intermediate precision	3.0 %	1.0 %

Method comparison

Standard application (IGA)

IgA values for human serum and plasma samples obtained on a COBAS INTEGRA 700 analyzer using the COBAS INTEGRA Immunoglobulin A reagent (sample predilution 1:21) (y) were compared with those determined using the commercially available reagents for IgA on a COBAS INTEGRA 700 analyzer (sample predilution 1:41) (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	Alternative system
Sample size	(n)	584	584
Corr. coefficient	(r)	0.998	0.994
	(r_s)	0.998	0.995
Linear regression		y = 1.003x + 0.078 g/L	y = 1.023x - 0.214 g/L
Passing/Bablok[12]		y = 1.008x + 0.069 g/L	y = 1.026x - 0.208 g/L

The sample concentrations were between 0.46 and 5.77 g/L (2.88-36.06 µmol/L and 46-577 mg/dL).

Sensitive application (IGAP)

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

		COBAS MIRA analyzer	Alternative system
Sample size	(n)	204	204
Corr. coefficient	(r)	0.996	0.996
	(r_s)	0.992	0.995
Linear regression		y = 1.043x + 0.041 g/L	y = 1.008x + 0.015 g/L
Passing/Bablok[12]		y = 1.142x - 0.006 g/L	y = 1.086x - 0.023 g/L

The sample concentrations were between 0.12 and 2.91 g/L (0.75-18.19 µmol/L and 12-291 mg/dL).

References

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

5 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.

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

7 Johnson AM. A new international reference preparation for proteins in human serum. Arch Pathol Lab Med 1993;117:29-31.

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

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

10 Schumann G, Dati F. Vorläufige Referenzbereiche für 14 Proteine im Serum (für Erwachsene) nach Standardisierung immunchemischer Methoden unter Bezug auf das internationale Referenzmaterial CRM 470. Lab Med 1995;19:401-403.

11 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. - Reference values are adapted to IFCC/BCR/CAP reference preparation CRM 470 (RPPHS 91/0619) by applying a conversion factor of 0.83.

12 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 (for USA: see https://usdiagnostics.roche.com for definition of symbols used):

CONTENT	Contents of kit
REAGENT	Reagent
→	Volume after reconstitution or mixing
GTIN	Global Trade Item Number

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

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