APOBT

cobas®

Tina-quant Apolipoprotein B ver.2

Specific proteins

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
03032574 122	Tina-quant Apolipoprotein B ver.2 (100 tests)	System-ID 07 6569 4	COBAS INTEGRA 400 plus COBAS INTEGRA 800
12172623 122	Calibrator f.a.s. Lipids (3 × 1 mL)	System-ID 07 6570 8	
12172623 160	Calibrator f.a.s. Lipids (3 × 1 mL, for USA)	System-ID 07 6570 8	
10781827 122	Precinorm L (4 × 3 mL)	System-ID 07 9026 5	
11285874 122	Precipath L (4 × 3 mL)	System-ID 07 9500 3	
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	

English

System information

Test APOBT:

Test ID 0-036 on COBAS INTEGRA 400 plus analyzers

Test ID 0-569 on COBAS INTEGRA 800 analyzers

Intended use

In vitro test for the quantitative immunological determination of human apolipoprotein B in serum and plasma on COBAS INTEGRA systems.

Summary[1,2]

Apolipoproteins are the protein constituents of the lipoproteins. The lipoproteins are classified according to their ultracentrifugal flotation density. The liver synthesizes very low density lipoproteins (VLDL). These particles mainly contain triglycerides and cholesterol. In presence of lipoprotein lipase the triglycerides are hydrolyzed and LDL particles with a high proportion of cholesterol are formed. Apolipoprotein B is the major protein constituent of LDL. About one third of these LDL particles provide cholesterol to peripheral cells. The other two thirds are metabolized by the liver. LDL uptake in all these tissues occurs via LDL receptors. Apolipoprotein B levels increase in pregnancy, hypercholesteremia, LDL receptor defect, bile obstruction, hyperlipemia type II, and nephrotic syndrome. Apolipoprotein B levels decrease during liver disease, α-β-lipoproteinemia, sepsis, and under estrogen administration.

The combined determination of apolipoprotein A-1 and apolipoprotein B and the calculation of the apolipoprotein B/apolipoprotein A-1 ratio can reflect a disorder of lipid metabolism and the risk of developing atherosclerosis and coronary heart disease particularly well providing an excellent addition to the classical HDL/LDL cholesterol determination. A high level of apolipoprotein A-1 (HDL) and a low level of apolipoprotein B (LDL) correlate best with a low risk for these diseases.

Test principle[3,4,5,6]

Immunoturbidimetric assay

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

Reagents - working solutions

R1 TRIS buffer: 50 mmol/L, pH 8.0; polyethylene glycol: 4.2 %; detergent; preservative (liquid).

SR Anti-human apolipoprotein B antibody (sheep): dependent on titer; TRIS buffer: 100 mmol/L, pH 8.0; preservative (liquid).

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.

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: Heparin (Li-, Na-, NH_4^+-) or EDTA (Na_2-, K_2-, K_3-) 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 1:21 (1+20) with NaCl solution by the instrument.

Centrifuge samples containing precipitates before performing the assay.

Stability:[7,8]	1 day at 15-25 °C
	8 days at 2-8 °C
	2 months at (-15)-(-20) °C (freeze only once)

Materials provided

See “Reagents – working solutions” section for reagents.

Materials required (but not provided)

NaCl Diluent 9 %, Cat. No. 20756350 322, system-ID 07 5635 0 for automatic postdilution and standard serial dilutions. NaCl Diluent 9 % is placed in its predefined rack position and is stable for 4 weeks on-board COBAS INTEGRA 400 plus/800 analyzers.

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.

APOBT

Tina-quant Apolipoprotein B ver.2

cobas®

Specific proteins

Application for serum and plasma

COBAS INTEGRA 400 plus test definition

Measuring mode	Absorbance
Abs. calculation mode	Endpoint
Reaction mode	D-R1-S-SR
Reaction direction	Increase
Wavelength A/B	340/659 nm
Calc. first/last	33/68
Typical prozone effect	> 6 g/L (> 600 mg/dL or > 11.7 µmol/L)
Antigen excess check	No
Predilution factor	21
Unit	g/L

Pipetting parameters

		Diluent (H_2O)
R1	100 µL	
Sample	6 µL	20 µL
SR	25 µL	10 µL
Total volume	161 µL	

COBAS INTEGRA 800 test definition

Measuring mode	Absorbance
Abs. calculation mode	Endpoint
Reaction mode	D-R1-S-SR
Reaction direction	Increase
Wavelength A/B	340/659 nm
Calc. first/last	44/96
Typical prozone effect	> 6 g/L (> 600 mg/dL or > 11.7 µmol/L)
Antigen excess check	No
Predilution factor	21
Unit	g/L

Pipetting parameters

		Diluent (H_2O)
R1	100 µL	
Sample	6 µL	20 µL
SR	25 µL	10 µL
Total volume	161 µL	

Calibration

Calibrator	Calibrator f.a.s. Lipids
Calibration dilution ratio	1:6, 1:10, 1:13, 1:35, 1:65 NaCl 0.9 % is used as zero calibrator performed automatically by the instrument.
Calibration mode	Logit/log 4
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 apolipoprotein B value of the undiluted calibrator, indicated in the package insert of the calibrator C.f.a.s. Lipids.

Traceability: This method has been standardized with regard to the IFCC reference preparation SP1-01 (October 1992 WHO-IRP) for apolipoprotein A-1 and the IFCC reference preparation SP3-07 for apolipoprotein B.[9,10,11,12]

Quality control

Reference range	Precinorm L or PreciControl ClinChem Multi 1
Pathological range	Precipath L or PreciControl ClinChem Multi 2
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[13]: g/L × 1.95[a)] = µmol/L

g/L × 100 = mg/dL

mg/dL × 0.0195[a)] = µmol/L

a) measured as B_{100}

Limitations - interference

Criterion: Recovery within ± 10 % of initial value.

Serum/plasma

Icterus:[14] 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:[14] No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 621 µmol/L or 1000 mg/dL).

Lipemia (Intralipid):[14] No significant interference up to an L index of 1000. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[15,16]

Rheumatoid factors: No significant interference.

In very rare cases, gammopathy, in particular type IgM (Waldenström’s macroglobulinemia), may cause unreliable results.[17]

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 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

0.20-4.0 g/L (0.39-7.8 µmol/L or 20-400 mg/dL) (typical measuring range)

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

Determine samples having lower concentrations via the rerun function. For samples with lower concentrations, the rerun function reduces the sample predilution factor to 10.5. Results from samples diluted using the rerun function are automatically multiplied by the reduced predilution factor.

Lower limits of measurement

Lower detection limit of the test:
0.20 g/L (0.39 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 = 21).

Expected values[18]

The following reference values were determined using serum from healthy adults:

Apolipoprotein B

Females n = 150	0.60-1.17 g/L (1.17-2.28 µmol/L or 60-117 mg/dL)
Males n = 150	0.66-1.33 g/L (1.29-2.59 µmol/L or 66-133 mg/dL)

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 (n = 21) and intermediate precision (1 aliquot per run, 1 run per day, 21 days). The following results were obtained:

	Level 1	Level 2
Mean	0.8 g/L (1.56 µmol/L or 80 mg/dL)	1.5 g/L (2.93 µmol/L or 150 mg/dL)
CV repeatability	1.2 %	1.1 %
CV intermediate precision	2.9 %	3.2 %

Method comparison

Apolipoprotein B values for human serum samples obtained on a COBAS INTEGRA 400 analyzer with the COBAS INTEGRA Tina-quant Apolipoprotein B ver.2 (APOBT) reagent (y) were compared with those determined on a COBAS INTEGRA 400 analyzer using the previous COBAS INTEGRA Apolipoprotein B (APOB) reagent (x), and with those obtained with the same reagent on a Roche/Hitachi 917 analyzer (x).

		COBAS INTEGRA 400 analyzer	Roche/Hitachi 917 analyzer
Sample size	(n)	93	105
Corr. coefficient	(r)	0.954	0.999
Linear regression		y = 0.997x + 0.084 g/L	y = 1.022x - 0.007 g/L
Passing/Bablok[19]		y = 1.020x + 0.029 g/L	y = 1.027x - 0.011 g/L

The sample concentrations were between 0.038 and 2.33 g/L (0.074 and 4.54 µmol/L or 3.80 and 233 mg/dL).

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

CONTENT	Contents of kit
→	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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Roche Diagnostics, Indianapolis, IN
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