LPA2

cobas®

Tina-quant Lipoprotein (a) Gen.2

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
05852625 190	Tina-quant Lipoprotein (a) Gen.2 (150 tests)	System-ID 07 7504 5	**cobas c** 311, **cobas c** 501/502

Materials required (but not provided):

05852641 190	Preciset Lp(a) Gen.2 (⟶ 5 x 1 mL)	Codes 962-966	
05852650 190	PreciControl Lp(a) Gen.2 (Level Low ⟶ 2 x 1 mL) PreciControl Lp(a) Gen.2 (Level High ⟶ 2 x 1 mL)	Code 137 Code 138	
04489357 190	Diluent NaCl 9 % (50 mL)	System-ID 07 6869 3	

English

System information

For **cobas c** 311/501 analyzers:

LPA2: ACN 723

For **cobas c** 502 analyzer:

LPA2: ACN 8723

Intended use

In vitro test for the quantitative determination of lipoprotein (a) in human serum and plasma on Roche/Hitachi **cobas c** systems.

Summary

Lipoprotein (a) is composed of an LDL-like particle to which the lipoprotein (a)-specific apolipoprotein (a) is bonded by a disulfide bridge. Apolipoprotein (a) is highly homologous to plasminogen. Lipoprotein (a) is a cholesterol-rich lipoprotein which is synthesized in the liver independently of triglycerides and is not subject to the influence of age or diet.[1]

Several unrelated studies showed that Lp(a) is an independent prospective risk factor for coronary heart disease. However acceptance is limited due to the fact that it is difficult to compare Lp(a) results between different clinical studies and the assays used showed strong variations and miscellaneous standardization levels.[2,3]

Main problem for accurate detection of Lp(a) is the size polymorphism of apolipoprotein a (apo (a)). Levels of Lp(a) vary drastically among individuals and ethnic groups as the level is predominantly determined by the apo (a) gene on chromosome 6.[4,5]

The high variable number of KRINGLE 4 type2 domains results in an apo (a) size ranging from 187 kDa to over 662 kDa. Assays with antibodies directed against this variable part of the Lp(a) molecule will underestimate Lp(a) in patients with apo (a) smaller than in the used calibrator and overestimate Lp(a) in such samples with larger apo (a) particles as in the calibrator. Based on the size heterogeneity it makes no sense to measure Lp(a) mass. Therefore the values should be expressed in terms of nanomoles per liter of Lp(a) protein.

Only standardization of these assays against an apo (a) size independent method will yield correct results. Such methods use antibodies that recognize a single copy of apo (a) per particle. By using the WHO/IFCC International Reference Reagent (SRM2B) this goal can be reached.[6] The value in this material has been assigned by using two different ELISA's based on monoclonal antibodies specific to two different unique epitopes present in apo (a).[7,8] High lipoprotein (a) concentrations in serum correlate with premature manifestation of atherosclerosis and strokes. When lipoprotein (a) concentrations exceed 75 nmol/L, the coronary risk is approximately doubled. In combination with elevated LDL-cholesterol concentrations, the risk increases approximately 6-fold. An elevated lipoprotein (a) level is considered to be the most sensitive parameter for the development of coronary heart disease, irrespective of other plasma lipoproteins. Lipoprotein (a) should be determined together with total cholesterol, HDL-cholesterol and LDL-cholesterol as well as triglycerides when assessing the total arteriosclerotic risk.

According to the European Atherosclerosis Society Lp(a) measurement should be recommended in selected cases at high risk and in subjects with a family history of premature cardio vascular disease.[9]

Test principle

Particle enhanced immunoturbidimetric assay.[10] Human lipoprotein (a) agglutinates with latex particles coated with anti-Lp(a) antibodies. The precipitate is determined turbidimetrically at 800 / 660 nm.

Reagents - working solutions

R1 Glycine buffer: 170 mmol/L, pH 7.0; stabilizers; BSA; rabbit serum 0.1 %, preservative

R3 Latex particles coated with polyclonal anti-human lipoprotein(a) antibodies (rabbit); glycine buffer: 170 mmol/L, pH 7.3, BSA; 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.

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

LPA2

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	6 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

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 K_2-EDTA and K_3-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.

With K_3-EDTA tubes pay particular attention that the tubes are adequately filled.

Centrifuge samples containing precipitates before performing the assay.

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

Stability:

If samples are not assayed within 8 hours, samples should be stored at 2-8 °C.[11]

If samples are not assayed within 48 h,[11] samples should be stored frozen at -70 °C or below.[12,13] Frozen samples should be thawed only once.

Analyte deterioration may occur in samples that are repeatedly frozen and thawed.

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 serum and plasma

cobas c 311 **test definition**

Assay type	2-Point End		
Reaction time / Assay points	10 / 26-49		
Wavelength (sub/main)	800 / 660 nm		
Reaction direction	Increase		
Unit	nmol/L		
Reagent pipetting		Diluent (H_2O)	
R1	133 µL	–	
R3	33 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	2.0 µL	0 µL	0 µL
Decreased	8.0 µL	10 µL	110 µL
Increased	2.0 µL	0 µL	0 µL

cobas c 501 **test definition**

Assay type	2-Point End		
Reaction time / Assay points	10 / 40-60		
Wavelength (sub/main)	800 / 660 nm		
Reaction direction	Increase		
Unit	nmol/L		
Reagent pipetting		Diluent (H_2O)	
R1	133 µL	–	
R3	33 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	2.0 µL	0 µL	0 µL
Decreased	8.0 µL	10 µL	110 µL
Increased	2.0 µL	0 µL	0 µL

cobas c 502 **test definition**

Assay type	2-Point End		
Reaction time / Assay points	10 / 40-60		
Wavelength (sub/main)	800 / 660 nm		
Reaction direction	Increase		
Unit	nmol/L		
Reagent pipetting		Diluent (H_2O)	
R1	133 µL	–	
R3	33 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	2.0 µL	0 µL	0 µL
Decreased	8.0 µL	10 µL	110 µL
Increased	4.0 µL	0 µL	0 µL

Calibration

Calibrators	S1: H_2O S2-S6: Preciset Lp(a) Gen.2
Calibration mode	Spline
Calibration frequency	Full calibration - after reagent lot change - as required following quality control procedures

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

Traceability: This method has been standardized against the IFCC reference material SRM2B for nmol/L.[14]

Quality control

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 c systems automatically calculate the analyte concentration of each sample.

Conversion factor:[15] mg/dL = (nmol/L + 3.83) × 0.4587

Limitations – interference

Criterion: Recovery within ± 6 nmol/L of initial values of samples ≤ 60 nmol/L and within ± 10 % for samples > 60 nmol/L.

Icterus:[16] 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:[16] No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 621 µmol/L or 1000 mg/dL).

Lipemia (Intralipid):[16] No significant interference up to an L index of 2000. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Rheumatoid factors: No significant interference up to a level of 1200 IU/mL.

Plasminogen: No significant cross-reactivity in the tested concentration range (up to 150 mg/dL).

Apolipoprotein B: No significant cross-reactivity in the tested concentration range (up to 200 mg/dL).

Drugs: No interference was found at therapeutic concentrations using common drug panels.[17, 18]

High dose hook-effect: No false result occurs up to a lipoprotein (a) concentration of 450 nmol/L.

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[19]

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

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ACTION REQUIRED

Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on **cobas c** systems. The latest version of the carry-over evasion list can be found with 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 required in certain cases.

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

Limits and ranges

Measuring range

Measuring range: 7-240 nmol/L

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.

Lower limits of measurement

Limit of Blank	= 6 nmol/L
Limit of Detection	= 7 nmol/L
Limit of Quantitation	= 20 nmol/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 30 %. It has been determined using low concentration Lp(a) samples.

Expected values

A Lp(a) concentration of 30 mg/dL corresponding to the 75th percentile in a male Caucasian reference population is widely used as cut-off point or threshold value.[20,21]

The European Atherosclerosis Society recommends screening for elevated Lp(a) in those at intermediate or high CVD/CHD risk and defines a desirable Lp(a) level ≤ 50 mg/dL.[22]

However the NHLBI recommends to stop using data for total Lp(a) mass, and to use nmol/L units instead, which consider the number of particles. Additionally they recommend to use assays independent from apo(a) size and standardized according to the IFCC reference material SRM2B.[23]

Based on the evaluation of Framingham data values above 75 nmol/L are regarded as a cut-off value for the presence of an increased risk.[23]

Elevated Lp(a) levels can be found in most racial/ethnicity groups, with the prevalence being lowest in whites and Asians. The median Lp(a) levels in black subjects and in Asian Indians from southern locations are 2- to 4-fold higher compared with whites, and up to 68 % of blacks have Lp(a) levels > 75 nmol/L, whereas levels above this threshold are present in around 25 % of whites.[24]

Therefore reference ranges have not been established for this assay for different ethnic populations or disease states. Since Lp(a) levels are largely influenced by hereditary factors and vary with ethnic populations it is recommended that each laboratory establish own expected values.

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:

	Repeatability		
Sample	Mean nmol/L	SD nmol/L	CV %
Lp (a) Control level L	40.7	0.6	1.5
Lp (a) Control level H	156	2	1.1
Human serum 1	18.2	1.0	5.6
Human serum 2	88.7	2.2	2.5
Human serum 3	226	2	0.8

	Intermediate precision		
Sample	Mean nmol/L	SD nmol/L	CV %
Lp (a) Control level L	40.7	0.7	1.7
Lp (a) Control level H	156	2	1.2
Human serum 1	18.2	1.5	8.0
Human serum 2	88.7	2.7	3.0
Human serum 3	226	2	1.1

The data obtained on **cobas c** 501 analyzer(s) are representative for **cobas c** 311 analyzer(s).

Method comparison

Lipoprotein (a) values for human serum and plasma samples obtained on a **cobas c** 501 analyzer (y) were compared with those determined using the corresponding reagent on COBAS INTEGRA 800 analyzers (x).

Sample size (n) = 240

Passing/Bablok[25]	Linear regression
y = 0.980x - 0.285 nmol/L	y = 0.985x - 0.745 nmol/L
τ = 0.938	r = 0.999

The sample concentrations were between 7.61 and 234 nmol/L.

Lipoprotein (a) values for human serum and plasma samples obtained on a **cobas c** 501 analyzer (y) were compared with those determined using the Northwest Lipid Metabolism and Diabetes Research Laboratories ELISA method traceable to the WHO/IFCC reference material SRM2B (x).

Sample size (n) = 105

Passing/Bablok[25]	Linear regression
y = 0.935x + 0.606 nmol/L	y = 0.942x + 0.105 nmol/L
τ = 0.947	r = 0.995

The sample concentrations were between 7.10 and 218 nmol/L.

The data obtained on **cobas c** 501 analyzer(s) are representative for **cobas c** 311 analyzer(s).

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.

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

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

CE 0123

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