LDL Colesterol

monofase AA

For LDL cholesterol determination in serum or plasma

SUMMARY

Plasma lipoproteins are spherical particles containing varying amounts of cholesterol, triglycerides, phospholipids and proteins. These particles solubilize and transport cholesterol into the bloodstream.

The relative proportion of protein and lipid determines the density of these lipoproteins and provides the basis to establish a classification. These classes are chylomicrons, very low-density lipoproteins (VLDL), low-density lipoproteins (LDL) and high-density lipoproteins (HDL). Numerous clinical studies have shown that the different lipoproteins classes have very distinct and varied effects on coronary heart disease risk. Such studies point out the LDL cholesterol as the key factor for the atherosclerosis pathogenesis and coronary heart disease (CHD), while HDL cholesterol is considered as a protective factor. An increase in LDL cholesterol may occur, even with normal cholesterol concentrations, associated to an increase in the CHD risk.

PRINCIPLE

The method consists in a two-step homogeneous assay without precipitation. In the first step, a surfactant is added (Reagent A) solubilizing non-LDL lipoprotein particles. The released cholesterol is consumed by cholesterol esterase and cholesterol oxidase in a reaction without color development. A second surfactant (Reagent B) solubilizes LDL particles forming a color proportional to the LDL cholesterol quantity present in sample, even in normal cholesterol concentrations.

PROVIDED REAGENTS

A. Reagent A: solution containing 1000 U/l cholesterol esterase, 1200 U/l cholesterol oxidase, 1250 U/l peroxidase, 3000 U/l abscorbate oxidase, 1 g/l 4-aminoantipyrine and 7 g/l surfactant in 50 mM MES buffer.

B. Reagent B: solution containing 0.4 g/l N,N-bis-(4-sulphobutyl)-m-disodium toluidine (DSBmT) and 10 g/l surfactant in 50 mM MES buffer.

Calibrator: lyophilized human serum containing different types of lipoproteins, including LDL. The concentration changes from batch to batch (see titer on label).

INSTRUCTIONS FOR USE

Reagent A and B: ready to use.

Calibrator: reconstitute with distilled water volume indicated on the label. Cap the vial and let stand it for 5 minutes. Then dissolve the vial content by gentle shaking avoiding foam formation.

WARNINGS

- Reagents are for “in vitro” diagnostic use.
- Do not pipette by mouth.
- The calibrator has been tested for HBsAg, HCV and antibody against HIV 1/2 being found non-reactive. Nonetheless, it should be handled as capable of transmitting infections.
- Use the reagents according to the working procedures for clinical laboratories.
- The reagents and samples should be discarded according to the local regulations in force.

STABILITY AND STORAGE INSTRUCTIONS

Provided Reagents: are stable in refrigerator (2-10°C) until the expiration date shown on the box. Do not freeze. Once the reagents are opened, they are stable for 4 weeks in refrigerator (2-10°C).

Calibrator: stable in refrigerator (2-10°C) until the expiration date shown on the box. Once reconstituted it is stable for 2 weeks in refrigerator (2-10°C). It may be aliquoted stored at -80°C.

SAMPLE

Serum or plasma

a) Collection: obtain the serum in the usual way.

b) Additives: in case plasma is used as sample, use EDTA or heparin as anticoagulant.

c) Known interfering substances: no interferences are observed by ascorbic acid up to 50 mg/dl, hemoglobin up to 500 mg/dl, bilirubin up to 20 mg/dl and γ-globulin up to 50 g/l. For samples with higher concentrations of interfering substances, dilute with saline solution before testing, multiplying the obtained result by the performed dilution.

See Young, D.S. in References for effect of drugs on the present method.

d) Stability and storage instructions: centrifuge and separate serum from clot within 3 hours after collection. In case the test could not be performed immediately, the sample can be kept for 5 days in refrigerator (2-10°C).

REQUIRED MATERIAL (non-provided)

- Volumetric material for measuring the stated volumes.
- Autoanalyzer.

PROCEDURE

(autoanalyzers)

A general procedure for LDL Colesterol monofase AA in an autoanalyzer is detailed below. When implementing the technique for a particular autoanalyzer, follow its working instructions.

Sample or **Calibrator**	3 ul
Reagent A	300 ul
Incubate for 5 minutes at 37ºC. Read the absorbance at 660/546 nm (Sample Blank).	
Reagent B	100 ul
Incubate for 5 minutes at 37ºC. Read the result at 660/546 nm (LDL-cholesterol concentration).	

CALIBRATION
Process the Calibrator in the same way as the samples. The Calibrator concentrations are near medical decision levels and change from batch to batch (see titer on label). Set the calibrator's concentration value every time the batch is changed.

CALCULATIONS
LDL Cholesterol (mmol/l) **=** LDL Cholesterol (mg/dl) x 0.02586

QUALITY CONTROL METHOD
Each time the test is performed, analyze two levels of a quality control material **(Standatrol S-E 2 niveles**) with known LDL cholesterol concentration.

REFERENCE VALUES
The expert panel from the National Cholesterol Education Program (NCEP) provides the following LDL Cholesterol values related to the risk of acquiring coronary heart disease (CHD):

- **Low or no risk** (normal individuals): LDL Cholesterol values below 129 mg/dl.
- **Moderate or high risk** (individuals with probability of acquiring CHD): values between 130 and 189 mg/dl.
- **Very High risk** (individuals suspected from suffering from CHD): LDL Cholesterol values ≥ 190 mg/dl.

However, each laboratory should establish its own references values.

PROCEDURE LIMITATIONS
See Known interfering substances under SAMPLE.
Anticoagulants containing citrate should not be employed.

PERFORMANCE
a) Accuracy: the method's accuracy was verified by comparison with the values obtained by the ultracentrifugation and cholesterol analysis reference method and with the LDL immuno-separation direct method. The comparison results were the following:

Method	LDL Colesterol monofase AA	Reference
Nº of samples	54	54
average	122.5 mg/dl	125.1 mg/dl
standard deviation	30.7 mg/dl	30.9 mg/dl
correlation coefficient: 0.96		

Method	LDL Colesterol monofase AA	Reference
Nº of samples	92	92
average	120.0 mg/dl	122.8 mg/dl
standard deviation	30.5 mg/dl	31.6 mg/dl
correlation coefficient: 0.97		

b) Precision: simultaneously processing 20 samples on the same day, the following intra-assay variation was obtained:

Level	S.D.	C.V.
98.1 mg/dl	± 0.72 mg/dl	0.73 %
146.5 mg/dl	± 0.96 mg/dl	0.66 %
209.8 mg/dl	± 1.31 mg/dl	0.62%

c) Detection limit: 0.278 mg/dl.

PARAMETERS FOR AUTOANALYZERS
For programming instructions check the user manual of the autoanalyzer in use.

WIENER LAB. PROVIDES
- 80 ml (1 x 60 ml + 1 x 20 ml), with Calibrator (Cat. Nº 1220220)
- 80 ml (2 x 30 ml + 2 x 10 ml), with Calibrator (Cat. Nº 1009283)
- 80 ml (2 x 30 ml + 2 x 10 ml), with Calibrator (Cat. Nº 1009348)
- 160 ml (2 x 60 ml + 2 x 20 ml), with Calibrator (Cat. Nº 1009627)

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Symbols

The following symbols are used in packaging for Wiener lab. diagnostic reagent kits.

CE — This product fulfills the requirements of the European Directive 98/79 EC for "in vitro" diagnostic medical devices

EC REP — Authorized representative in the European Community

IVD — "In vitro" diagnostic medical device

Σ — Conteins sufficient for <n> tests

Use by

Temperature limitation (store at)

Do not freeze

Biological risks

Volume after reconstitution

Cont. — Contents

LOT — Batch code

Manufactured by:

Xn — Harmful

Corrosive / Caustic

Xi — Irritant

Consult instructions for use

Calibr. — Calibrator

CONTROL — Control

CONTROL + — Positive Control

CONTROL − — Negative Control

REF — Catalog number

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