



HDL Cholesterol

fast

Homogeneous colorimetric method for HDL-cholesterol determination in serum or plasma

SUMMARY

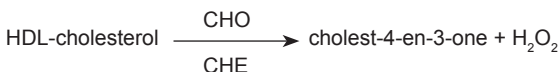
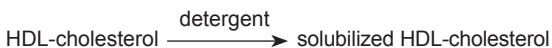
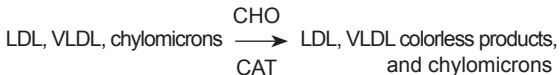
Plasma lipoproteins are spherical particles containing varying amounts of cholesterol, triglycerides, phospholipids and proteins. Phospholipids, free cholesterol and proteins constitute the outer surface of the lipoprotein particle, while the inner core contains mostly esterified cholesterol and triglycerides. 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. The classes are: chylomicron, very-low-density lipoproteins (VLDL), low-density lipoproteins (LDL) and high-density lipoproteins (HDL). Numerous clinical studies have shown that the different lipoprotein classes have very distinct and varied effects on coronary heart disease risk.

The main role of HDL in lipid metabolism is the uptake and transport of cholesterol from peripheral tissues to the liver through a process known as reverse cholesterol transport (cardioprotective mechanism).

Low HDL-cholesterol levels are associated with an increased risk of coronary heart disease. Hence, the determination of serum HDL-cholesterol is a useful tool for identifying high-risk patients.

PRINCIPLE

The present is a homogeneous method using two reagents for HDL-cholesterol determination. During the first reaction stage, free cholesterol or cholesterol bound to proteins different from HDL is solubilized and consumed in a reaction involving cholesterol oxidase (CHO) and catalase (CAT), yielding a non-colored product. During a second stage, a particular agent (azide) blocks CAT action and a detergent specifically solubilizes HDL. The HDL-cholesterol is released to react with cholesterol esterase (CHE), CHO, 4-AAP (4-aminoantipyrene) and N-ethyl-N-(2-hydroxy-3-sulfopropyl)-3-toluidine disodium (TOOS), yielding a colored product, which may be read at 540-600 nm.



PROVIDED REAGENTS

A. Reagent A: cholesterol oxidase solution (< 3000 U/l), peroxidase (< 5000 U/l), catalase (< 3000 U/l) and N-ethyl-N-(2-hydroxy-3 sulfopropyl)-3-toluidine disodium (TOOS) (< 1 mM) in Good buffer with appropriate stabilizer and preservative.

B. Reagent B: detergent solution (< 2%), cholesterol esterase (< 3000 U/l) and 4-aminoantipyrene (4-AAP) (< 1 mM) in Good buffer with sodium azide (0.9 g/l) and appropriate stabilizer.

Calibrator*: lyophilized human serum containing various types of lipoproteins including HDL. The concentration varies from batch to batch (see title on the label).

NON-PROVIDED REAGENTS

- Distilled water.
- **HDL Cholesterol Calibrator** (for package sizes without Calibrator).

INSTRUCTIONS FOR USE

Reagents A and B: ready to use.

Calibrator: reconstitute with the volume of distilled water stated on the label. Replace the stopper and let stand for 5 minutes. Dissolve by gently rotating the vial avoiding foam formation. Do not shake.

WARNINGS

- The reagents are for "in vitro" diagnostic use.
- Do not pipette by mouth.
- The calibrator has been tested for HBsAg, HCV and antibodies to HIV 1/2, being nonreactive. However, it should be processed as if they were infectious material.
- Use the reagents according to the working procedures for clinical laboratories.
- All reagents and samples should be discarded according to local regulations.

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 opened, the reagents are stable for 3 weeks in refrigerator (2-10°C).

After reconstitution, the Calibrator is stable for 1 week in refrigerator (2-10°C) or for 1 month frozen (-20°C), avoiding thawing and refreezing.

SAMPLE

Serum or plasma

* Non-provided with some kit sizes

- a) Collection:** obtain the sample in the usual way.
- b) Additives:** heparin or EDTA when plasma is used as sample.
- c) Known interfering substances:** no interference is observed by ascorbic acid up to 24 mg/dl, hemoglobin up to 1000 mg/dl, bilirubin up to 8.0 mg/dl or triglycerides up to 3000 mg/dl (see PROCEDURE LIMITATIONS). Refer to the literature of Young for the effects of drugs on the present method.
- c) Stability and storage instructions:** centrifuge and separate the serum from the clot within 3 hours of extraction. If samples not processed immediately, they can be stored for 1 week in refrigerator (2-10°C).

MATERIAL REQUIRED (not provided)

- Volumetric material for measuring the stated volumes
- Autoanalyzer

PROCEDURE
(Automated analyzers)

A general procedure for **HDL Cholesterol fast** in an automatic analyzer is detailed. When the technique is implemented for a particular analyzer, follow its work instructions.

Sample or Calibrator	3 ul
Reagent A	300 ul

Incubate for 5 minutes at 37°C. Read the absorbance at 540-600 nm (Sample Blank).

Reagent B	100 ul
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Incubate for 5 minutes at 37°C. Read the result at 540-600 nm (HDL-cholesterol).

CALIBRATION

The Calibrator must be processed in the same manner as the samples. Calibrator concentrations are close to medical decision levels and vary batch to batch (see title on the label). Enter the concentration value of the calibrator each time a batch is changed.

QUALITY CONTROL METHOD

Process 2 levels of a quality control material (**Standatrol S-E 2 niveles**) with known concentrations of HDL-cholesterol, with each determination.

REFERENCE VALUES

The expected values of HDL-cholesterol are:
Men: 30-70 mg/dl
Women: 30-85 mg/dl

The expert panel of the National Cholesterol Education Program (NCEP) provides the following values of HDL cholesterol: 40 - 60 mg/dl

It is recommended that each laboratory establish its own reference values. However, values greater than 40 mg/dL are

considered desirable and those that are above 60 mg/dl were considered protective. By contrast, HDL-cholesterol values below 40 mg/dl are considered as significant risk of coronary heart disease.

PROCEDURE LIMITATIONS

Anticoagulants containing citrate should not be used. Do not expose reagents to light. Preserve the reagents according to the instructions. For samples with triglyceride levels above 3000 mg/dl, dilute with saline.

PERFORMANCE

a) Precision: simultaneously processing replicates of the same sample on the same day, the following values were obtained:

Level	DS	CV
24.1 mg/dl	± 0.20 mg/dl	0.8%
50.9 mg/dl	± 0.34 mg/dl	0.7%
75.9 mg/dl	± 0.93 mg/dl	1.2%

Processing the same sample on different days the following values were obtained:

Level	DS	CV
24.1 mg/dl	± 0.49 mg/dl	2.0%
50.9 mg/dl	± 0.45 mg/dl	0.9%
75.9 mg/dl	± 1.84 mg/dl	2.3%

b) Linearity: the reaction is linear to 150 mg/dl. For higher values, dilute the sample with saline solution and multiply the result by the dilution factor used.

c) Quantification limit: the minimum quantifiable concentration of HDL-cholesterol is 4 mg/dl.

d) Recovery: adding known quantities of HDL-cholesterol to different sera, a recovery between 98.4 and 99.0% was obtained.

PARAMETERS FOR AUTOMATIC ANALYZERS

For programming instructions refer to the User Manual of the analyzer in use.

WIENER LAB. PROVIDES

40 ml: - 1 x 30 ml Reagent A
- 1 x 10 ml Reagent B
(Code 1220229)

80 ml: - 1 x 60 ml Reagent A
- 1 x 20 ml Reagent B
(Code 1220233)


160 ml: - 2 x 60 ml Reagent A
- 2 x 20 ml Reagent B
- 1 x 1 ml Calibrator
(Code 1009691)

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SYMBOLS

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

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

 Authorized representative in the European Community

 "In vitro" diagnostic medical device

 Contains sufficient for <n> tests

 Use by

 Temperature limitation (store at)

 Do not freeze

 Biological risks

 Volume after reconstitution

 Contents

 Batch code

 Manufactured by:

 Harmful

 Corrosive / Caustic

 Irritant

 Consult instructions for use


 Calibrator

 Control

 Positive Control

 Negative Control

 Catalog number

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