# HDL Colesterol

## *monofase AA plus*

**Colorimetric method without precipitation for the determination of HDL-cholesterol 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. 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), peroxidase (POD) and N-ethyl-N-(2-hydroxy-3-sulphopropyl)-3-dissodium toluidine (TOOS) yielding a non-colored product. During a second stage, a surfactant specifically solubilizes HDL. The HDL-cholesterol is released to react with cholesterol esterase (CHE), cholesterol oxidase and TOOS, yielding a colored product:

$$\text{LDL, VLDL, chylomicrons} \xrightarrow[\text{CHO}]{\text{TOOS}} \text{non-colored products from LDL, VLDL and chylomicrons}$$

$$\text{HDL-cholesterol} \xrightarrow{\text{surfactant}} \text{solubilized HDL}$$

$$\text{HDL-cholesterol} \xrightarrow[\text{CHE}]{\text{CHO}} \text{cholest-4-en-3-ona} + H_2O_2$$

$$H_2O_2 + \text{TOOS} + \text{4-AAP} \xrightarrow{\text{POD}} \text{color development}$$

**PROVIDED REAGENTS**

**A. Reagent A:** cholesterol oxidase solution (< 1000 U/l), peroxidase (< 1300 U/l) and N-ethyl-N-(2-hydroxy-3-sulphopropyl)-3-dissodium toluidine (TOOS) (< 1 mM) in Good buffer with appropriate stabilizing agent and preservative.

**B. Reagent B:** surfactant solution (< 2%), cholesterol esterase (< 1500 U/l) and 4-aminoantipyrine (4-AAP) (< 1 mM) in Good buffer, with appropriate stabilizing agent and preservative.

**Calibrator*:** lyophilized human serum containing different types of lipoproteins including HDL. Concentration varies batch to batch (see titer on label).

**NON-PROVIDED REAGENTS**

Distilled water.

**INSTRUCTIONS FOR USE**

**Reagents A and B:** ready to use.

**Calibrator:** reconstitute with the volume of distilled water indicated on the label. Cap the vial and let stand for 5 minutes. Help dissolution by gently rotating the vial, avoiding foam formation. Do not shake.

**WARNINGS**

- Reagents are for "in vitro" diagnostic use.
- Do not pipette by mouth.
- The Calibrator has been tested and found non-reactive for HBsAg, HCV and antibodies against HIV 1/2. Nevertheless, they should be treated as if capable of transmitting infection.
- 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 opened, reagents are stable for 3 weeks in refrigerator (2-10°C).

Once reconstituted, the Calibrator stable for 1 week in refrigerator (2-10°C) of for 1 month frozen (-20°C), avoiding repeated freezing and thawing.

**SAMPLE**

Serum or plasma

**a) Collection:** obtain sample in the usual way.

**b) Additives:** use heparin or EDTA when plasma is used as sample.

**c) Known interfering substances:** no interferences have been observed from ascorbic acid up to 100 mg/dl, hemoglobin up to 1000 mg/dl, bilirubin up to 60 mg/dl and triglycerides up to 1200 mg/dl (see PROCEDURE LIMITATIONS).

** Non-provided with some of kit sizes*

Refer to Young, D.S. in References for drugs' effect on the present method.
**d) Stability and storage instructions:** centrifuge and separate serum from clot within 3 hours of extraction. If samples are not processed immediately they can be stored for 1 week in refrigerator (2-10°C).

**REQUIRED MATERIAL** (non-provided)
- Volumetric material appropriate for measuring stated volumes.
- Autoanalyzer.

**PROCEDURE**
(autoanalyzers)
Below is a general example of the **HDL Colesterol monofase AA plus** test procedure for an autoanalyzer. Whenever a technique for a particular analyzer is performed, follow its working instructions.

| | |
|---|---|
| **Sample** or **Calibrator** | 3 ul |
| **Reagent A** | 300 ul |
| Incubate for 5 minutes at 37°C. Read absorbance at 600/700 nm (Sample Blank). | |
| **Reagent B** | 100 ul |
| Incubate for 5 minutes at 37°C. Read result at 600/700 nm (HDL-cholesterol concentration). | |

**CALIBRATION**
The Calibrator should be processed together with and in the same way as the samples. Calibrator concentrations are close to medical decision levels and vary from batch to batch (see titer on label). Enter the calibrator's concentration value every time that a batch is replaced.

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

**REFERENCE VALUES**
Expected HDL cholesterol values are the following:
Men: 30 - 70 mg/dl
Women: 30 - 85 mg/dl
The National Cholesterol Education Program (NCEP) Expert Panel provided the following references values:
40 - 60 mg/dl
It is recommended that each laboratory establish its own reference values. However, values over 40 mg/dl are recommended. Values over 60 mg/dl have been regarded as protective. On the contrary, HDL-cholesterol values below 40 mg/dl are considered as an indicator of coronary heart disease risk.

**PROCEDURE LIMITATIONS**
Anticoagulants containing citrate should not be used. Protect the reagents from direct sunlight. Store reagents according to the instructions. In case of using samples with triglycerides concentration over 1200 mg/dl, dilute the sample with saline solution.

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

| Level | S.D. | C.V. |
|---|---|---|
| 32.9 mg/dl | ± 0.6 mg/dl | 1.9 % |
| 50.7 mg/dl | ± 0.9 mg/dl | 1.7 % |
| 101.3 mg/dl | ± 1.5 mg/dl | 1.5 % |

Processing the same sample on different days, the following values are obtained:

| Level | S.D. | C.V. |
|---|---|---|
| 32.8 mg/dl | ± 0.8 mg/dl | 2.4 % |
| 50.0 mg/dl | ± 1.2 mg/dl | 2.5 % |
| 100.1 mg/dl | ± 2.3 mg/dl | 2.3 % |

**b) Linearity:** reaction is linear up to 200 mg/dl. For higher values dilute the sample with saline solution and multiply the result by the dilution factor used.
**c) Detection limit:** the minimum measurable HDL cholesterol concentration is 4 mg/dl.
**d) Recovery:** by adding known amounts of HDL cholesterol to different sera, it was observed that recovery ranged between 98.4 and 99.0%.

**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. 1220223)
- 80 ml (1 x 60 ml + 1 x 20 ml), without Calibr. (Cat. 1220224)
- 80 ml (2 x 30 ml + 2 x 10 ml), with Calibrator (Cat. 1009328)
- 80 ml (2 x 30 ml + 2 x 10 ml), with Calibrator (Cat. 1009280)
- 160 ml (2 x 60 ml + 2 x 20 ml), with Calibrator (Cat. 1009622)

**REFERENCES**

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- Tietz N.W., Clinical Guide to Laboratory Tests, W.B. Saunder Co., Philadelphia, pag. 256, 1986.
- Westgard, J. et al. - Clin. Chem. 20:825 (1974).

# 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 | Contains 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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