SUMMARY
Plasma lipoproteins are globular particles that contain varying amounts of cholesterol, triglycerides, phospholipids and proteins. Phospholipids, free cholesterol and proteins constitute the outer surface of the lipoprotein particle, while its core contains in greater proportion esterified cholesterol and triglycerides. These particles solubilize and transport cholesterol in the bloodstream. Lipoprotein proteins can have different functions: stabilization of the structure, cellular recognition in peripheral tissues, and interrelation with other lipoproteins allowing the exchange of material between them. The relative proportion of protein and lipid determines the density of these lipoproteins and provides the basis to establish a classification. The 4 most important classes are (in order of increasing density): chylomicrons, very low density lipoproteins (VLDL), low density lipoproteins (LDL) and high density lipoproteins (HDL). Numerous clinical studies have shown that different classes of lipoproteins have different and varied effects on the risk of coronary heart disease. The main function of HDL in lipid metabolism is the uptake and transport of cholesterol from the peripheral tissues to the liver in a process known as reverse cholesterol transport (cardioprotective mechanism). HDL is much smaller than other lipoproteins and are most abundant from the numerical point of view. Its protein composition is 50%, which explains its high density compared to the others. The elevation of HDL cholesterol (HDL-c) could mean a higher reverse transport of cholesterol from the peripheral tissues to the liver, which would have an antiatherogenic effect, but it could also imply an alteration in the hepatic elimination, which would suggest a proatherogenic effect. Similarly, low HDL-c values may not indicate a problem in reverse cholesterol transport but a high liver clearance rate. Consequently the low concentration of HDL-c would not correlate with proatherogenic effects, but with antiatherogenic effects, as in the Apo Al Milano genotype. However, low HDL-C is associated with a high risk of heart disease. For this reason, HDL-cholesterol determination is a useful tool in the identification of high-risk individuals.

PRINCIPLE
Homogeneous bireagent method for the determination of HDL cholesterol (HDL-C). In the first stage of the reaction, free cholesterol associated with proteins other than HDL is solubilized and consumed in a reaction that involves cholesterol oxidase (CHO) and peroxidase (POD), yielding an uncolored product. In a second step, a detergent solution specifically solubilizes HDL. HDL-cholesterol is thus released to react with cholesterol esterase (CHE), CHO, 4-AAP (4-amino antipyrine) and N-ethyl-N-(2-hydroxy-3-sulfopropyl)-3-toluidine disodium (TOOS), yielding a colored product that is read at 540-600 nm.

POD
LDL, VLDL, chylomicrons → LDL, VLDL colorless products, CHO and chylomicrons

HDL-cholesterol → detergent sol. → solubilized HDL

HDL-cholesterol → CHO → cholest-4-en-3-one + H₂O₂

CHE

H₂O₂ + TOOS + 4-AAP → color development

PROVIDED REAGENTS
A. Reagent A: cholesterol oxidase solution (< 3000 U/l), peroxidase (< 5000 U/l), and N-ethyl-N-(2-hydroxy-3-sulfopropyl)-3-toluidine disodium (TOOS) (< 1 mM) in Good’s buffer with appropriate stabilizer and preservative.
B. Reagent B: detergent solution (< 2%), cholesterol esterase (< 3000 U/l) and 4-aminoantipyrine (4-AAP) (< 1 mM) in Good buffer, preservatives 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.

* Non-provided with some kit sizes
- 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 at least 4 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
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 25 mg/dl, hemoglobin up to 1000 mg/dl, bilirubin up to 50 mg/dl or triglycerides up to 3000 mg/dl (see PROCEDURE LIMITATIONS). Refer to Young’s bibliography 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).

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

<table>
<thead>
<tr>
<th>Level</th>
<th>DS</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>31.1 mg/dL</td>
<td>± 0.59 mg/dL</td>
<td>1.9%</td>
</tr>
<tr>
<td>48.1 mg/dL</td>
<td>± 0.33 mg/dL</td>
<td>0.7%</td>
</tr>
<tr>
<td>58.3 mg/dL</td>
<td>± 0.29 mg/dL</td>
<td>0.5%</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Level</th>
<th>DS</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>31.1 mg/dL</td>
<td>± 0.23 mg/dL</td>
<td>2.4%</td>
</tr>
<tr>
<td>48.1 mg/dL</td>
<td>± 0.67 mg/dL</td>
<td>1.4%</td>
</tr>
<tr>
<td>58.3 mg/dL</td>
<td>± 0.64 mg/dL</td>
<td>1.1%</td>
</tr>
</tbody>
</table>

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 3 mg/dl.
d) **Recovery:** adding known quantities of HDL-cholesterol to different sera, a recovery between 99.1% and 101.6% was obtained.

**PARAMETERS FOR AUTOMATIC ANALYZERS**
For programming instructions refer to the User Manual of the analyzer in use.

**WIENER LAB. PROVIDES**
- 80 ml (1 x 60 ml + 1 x 20 ml), with Calibrator (Code 1220231)
- 80 ml (1 x 60 ml + 1 x 20 ml), without Calibrator (Code 1220239)
REFERENCES

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

- 80 ml (2 x 30 ml + 2 x 10 ml), with Calibrator (Code 1009401)
- 80 ml (2 x 30 ml + 2 x 10 ml), with Calibrator (Code 1009264)
- 160 ml (2 x 60 ml + 2 x 20 ml), with Calibrator (Code 1009702)
- 160 ml (2 x 60 ml + 2 x 20 ml), with Calibrator (Code 1009930)

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