HDL cholesterol
Direct Enzymatic colorimetric
Quantitative determination
For In-Vitro and professional use only
Store at 2° to 8°C

INTENDED USE
For direct determination of human serum HDL cholesterol concentration

INTRODUCTION
HDL particles carry cholesterol from the cells back to the liver. HDL is known as “good cholesterol” because high levels are thought to lower the risk of heart disease. A low HDL cholesterol level is considered a greater heart disease risk. Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

PRINCIPLE OF THE METHOD
Directly determination of serum HDLc and (high-density lipoproteins cholesterol) levels without the need for any pre-treatment or centrifugation of the sample. The method depends on the properties of a detergent which solubilizes only the HDL so that the HDL-c is released to react with the cholesterol esterase, cholesterol oxidase and Chromogens to give color. The non HDL lipoproteins LDL, VLDL and chylomicrons are inhibited from reacting with the enzymes due to absorption of the detergents on their surfaces. The intensity of the color formed is proportional to the HDL-c concentration in the sample.

REAGENTS

<table>
<thead>
<tr>
<th>R 1</th>
<th>GOOD</th>
<th>Cholesterol oxidase</th>
<th>&lt;1000U/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>DSBmT</td>
<td>&lt;1mM</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>R 2</th>
<th>GOOD</th>
<th>PH 7.0</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Cholesterol esterase</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4-Aminoantipyrine (4-AP)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Detergent</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ascorbic oxidase</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Peroxidase</td>
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</table>

HDLc CAL
Calibrator : Lyophilized human serum.

PREPARATION
- The reagent is ready to use.
- Dissolve HDL/LDL Calibrator with 1ml of Distilled water.
- Mix thoroughly, avoiding foam forming.
- Bring to room temperature for about 30 min before use.
- Improper handling and/or storage can affect results.
- Inaccurate reconstitution and errors in assay technique can cause erroneous results.

STORAGE AND STABILITY
- All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8°C, and contaminations prevented during their use.
- Do not freeze the reagent
- Do not use reagents over the expiration date.
- After reconstitution, HDL/LDL calibrator is stable for:
  - R1/R2: Once opened is stable 8 weeks at 2-8°C
  - HDLc/LDLc CAL : Once reconstitute 2 weeks at 2-8°C or 3 months at -20°C
- Signs of reagent deterioration:
  - Presence of particles and turbidity.

EQUIPMENTS NEEDED BUT NOT PROVIDED
- Spectrophotometer or colorimeter measuring at 600 nm.
- Matched cuvettes 1.0 cm light path.
- General laboratory equipment.

SAMPLES
Serum or heparinized plasma: Free of Hemolysis. Removed from the blood clot as soon as possible.

Stability: HDL Cholesterol is stable for 7 days at 2-8°C.

PROCEDURE
1. Assay conditions:
   - Wavelength: 600-700 nm
   - Cuvette: 1 cm light path
   - Temperature: 37°C
2. Adjust the instrument to zero with distilled water.
3. Pipette into a cuvette:

<table>
<thead>
<tr>
<th>R1(µL)</th>
<th>Calibrator</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>300</td>
<td>300</td>
<td>300</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Sample (µL)</th>
<th>Calibrator</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>--</td>
<td>3</td>
<td>--</td>
</tr>
</tbody>
</table>

4. Mix well and incubate for 5 min at 37°C.
5. Read the absorbance (A1) of the sample and Calibrator
6. Add

<table>
<thead>
<tr>
<th>R2(µL)</th>
<th>Blank</th>
<th>Calibrator</th>
<th>Sample</th>
</tr>
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<tbody>
<tr>
<td>100</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

7. Mix well and incubate for 5 min at 37°C.
8. Read the absorbance (A2) of the sample and Calibrator, against the Blank.
9. Calculate the increase of the absorbance
   \[ A = A2 - A1 \]

CALCULATIONS

\[ (A) \text{ Sample } \times \text{ Calibrator (conc.)} = \text{mg/dL HDLc (in the (A)Calibrator sample)}. \]

CONVERSION FACTOR: mg/dL x0.0259 = mmol/L

QUALITY CONTROL
Control sera are recommended to monitor the performance of assay procedures. If control values are found outside the defined range, check the instrument, reagents and calibrator for problems. Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet
the acceptable tolerances.
HDL/LDL cholesterol calibrator values are: 45mg/dl
HDL Cholesterol Calibrator: 1.03mmol/l

REFERENCE VALUES

<table>
<thead>
<tr>
<th></th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lower risk</td>
<td>&gt; 50 mg/dl</td>
<td>&gt; 60 mg/dl</td>
</tr>
<tr>
<td>Standard risk</td>
<td>35-50 mg/dl</td>
<td>45-60 mg/dl</td>
</tr>
<tr>
<td>Increased risk</td>
<td>&lt; 35 mg/dl</td>
<td>&lt; 45 mg/dl</td>
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</tbody>
</table>

These values are for orientation purpose; each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS

Measuring range:
From detection limit of 2.5 mg/dL to linearity limit of 200 mg/dL.
If the results obtained were greater than linearity limit, dilute the sample 1/2 with NaCl 9 g/L and multiply the result by 2.

Precision:

<table>
<thead>
<tr>
<th></th>
<th>Intra-assay (n=20)</th>
<th>Inter-assay (n=20)</th>
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<tbody>
<tr>
<td>Mean (mg/dL)</td>
<td>32.9</td>
<td>32.8</td>
</tr>
<tr>
<td>SD</td>
<td>0.3</td>
<td>0.4</td>
</tr>
<tr>
<td>CV (%)</td>
<td>0.8</td>
<td>1.3</td>
</tr>
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Sensitivity:
1 mg/dL = 0.0016 A.

Accuracy:
Results obtained using ATLAS reagents (y) did not show systematic differences when compared with other commercial reagents (x).
The results obtained using 50 samples were the following:
Correlation coefficient (r): 0.996.
Regression equation: y = 0.98 x + 3.42
The results of the performance characteristics depend on the analyzer used.

INTERFERENCES

No interferences were observed to bilirubin T.and D up to 60 mg/dL
Hemoglobin up to 1000mg/dL or lipemia up to 1800 mg/dL.
A list of drugs and other interfering substances with HDL cholesterol determination has been reported by Young et. Al.

Notes
The reagent 2 presents yellowish coloration due to the peroxidaes, but it dose not affect its functionality.

REFERENCES


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