HDL CHOLESTEROL
Direct Enzymatic colorimetric, Liquid

Intended use
Spectrum diagnostics HDL cholesterol reagent is intended for in-vitro quantitative determination of HDL cholesterol in human serum, heparinized or EDTA plasma.

Background
HDL particles serve to transport in the blood-stream. HDL is known as “good cholesterol” because high levels are thought to lower the risk of heart disease and coronary artery 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.

METHOD
Direct Enzymatic colorimetric

PRINCIPLE
The assay is based on a modified poly vinyl sulfonic acid (PVS) and polyethylene-glycol-methyl ether (PEGME) coupled classic precipitation method with the improvements in using optimized quantities of PVS/PEGME and selected detergents, LDL, VLDL, and chyomicron (CM) react with PVS and PEGME and the reaction results in inaccessibility of LDL, VLDL and CM by cholesterol Oxidase (CHOD) and cholesterol esterase (CHER). The enzymes selectively react with HDL to produce H2O2 which is detected through a Tider reaction.

\[
\begin{align*}
\text{HDL} + \text{LDL} + \text{VLDL} + \text{CM} & \rightarrow \text{PVS} \\
\text{HDL} + (\text{LDL} + \text{VLDL} + \text{CM}) & \rightarrow \text{PEGME} \\
\text{HDL} + \text{CHOD} + \text{CHER} & \rightarrow \text{Fatty Acid + H2O2} \\
\text{H2O2 +4-AA+TODB} & \rightarrow \text{Quinone + 5 H2O} \\
\end{align*}
\]

REAGENTS
Reagents Composition

Reagent 1 (R1): MES buffer (pH 6.5), TODB N, N-Bis (4-sulfobutyl)-3-methylamine), Polyvinyl sulfonic acid, Polyethylene-glycol-methyl ester, MgCl2, Detergent, EDTA

Reagent 2 (R2): MES buffer (pH 6.5), Cholesterol esterase, Cholesterol Oxidase, Peroxidase, 4-aminoantipyrine, detergent.

HDL Calibrator
Standard, Lyophilized Human Serum HDL actual concentration is stated on the vial label.

Precautions
HDL Calibrator
Components from human origin have been tested and found to be negative for the presence of HBsAg, HCV and antibody to HIV (1/2). However handle cautiously as potentially infectious.

SAMPLE COLLECTION AND PRESERVATION
Serum or heparinized plasma, free of haemolysis; Anticoagulants containing citrate should not be used.

Removed from the blood clot as soon as possible. Stability of the sample: 7 days at 2-8°C.

REAGENT PREPARATION AND STORAGE
- R1 and R2: Are ready to use.
- HDL Calibrator: Dissolve the contents with distilled water, as mentioned on vial label. Cap vial and mix gently to dissolve contents. Wait for 30 minutes before use.
- R1 and R2: Once opened is stable 8 weeks at 2-8°C.
- HDL Calibrator: Once reconstituted 2 weeks at -20°C.
- Do not use reagents over the expiration date.
- Sign of reagent deterioration.
- Presence of particles and turbidity.

ADDITIONAL EQUIPMENT
- Spectrophotometer or colorimeter measuring at 600 nm.
- Matched cuvettes 1.0 cm light path.
- General laboratory equipment.

REAGENT 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 are prevented during their use. Do not freeze the reagents.

LINEARITY: 150 mg/dl

PROCEDURE
This reagent can be used manually (see method below) and on most analyzers. Applications are available on request.

Wavelength 600 nm (580 nm is an option)
Cuvette 1 cm
Temperature 37°C
Measure Against distilled water

<table>
<thead>
<tr>
<th></th>
<th>Blank</th>
<th>Calibrator</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1 (μL)</td>
<td>300</td>
<td>300</td>
<td>300</td>
</tr>
<tr>
<td>Calibrator(μL)</td>
<td>-</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>Sample (μL)</td>
<td>-</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>Mix and incubate for 5 min at 37°C. Then add : R2(μL)</td>
<td>100</td>
<td>100</td>
<td>100</td>
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</tbody>
</table>

Mix Read Immediately the absorbance (A1) of the samples and calibrator, against the Blank, then Read the absorbance (A2) of the Samples and calibrator after 5 mins, against the Blank. Calculate the Increase of the absorbance \( \Delta A = A_2 - A_1 \).
CALCULATION

\[(\Delta A) \text{ Sample} = \frac{X \text{ Calibrator conc.} = \text{mg/dL of HDL-C}}{(\Delta A) \text{ Calibrator}}\]

Conversion factor: \(\text{mg/dL} \times 0.0259 = \text{mmol/L}\)

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.

Expected Values

<table>
<thead>
<tr>
<th></th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low risk</td>
<td>&gt; 50 mg/dL</td>
<td>&gt; 60 mg/dL</td>
</tr>
<tr>
<td>Normal risk</td>
<td>35-50 mg/dL</td>
<td>45-60 mg/dL</td>
</tr>
<tr>
<td>High risk</td>
<td>&lt; 35 mg/dL</td>
<td>&lt; 45 mg/dL</td>
</tr>
</tbody>
</table>

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

Dynamic range:
The measuring range is from 1.0 mg/dL to linearity limit of 180 mg/dL. If the results obtained were greater than linearity limit, dilute the sample 2 times with NaCl 9 g/L and multiply the result by 2.

Sensitivity
The sensitivity of the test is 1 mg/dL

Accuracy
Results obtained using Spectrum reagents (y) did not show systematic difference when compared with other commercial reagents. (x).
The results obtained using 50 samples were the following.
Correction coefficient (r): 0.996.
Regression equation: \(y = 0.98 + 3.42 \text{ mg/dL}\).

INTERFERENCES

No Interferences were observed to bilirubin T. and D. up to 60 mg/dL Hemoglobin up to 1000 mg/dL or lipaemia up to 1800 mg/dL.

NOTES
Spectrum has Instrument application sheets for several automatic analyzers. Instructions for many of them are available on request.

BIBLIOGRAPHY

2. US National Cholesterol Education Program of the National Institutes of Health.

ORDERING INFORMATION

<table>
<thead>
<tr>
<th>CATALOG NO.</th>
<th>QUANTITY</th>
</tr>
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<tbody>
<tr>
<td>267 001</td>
<td>100 tests</td>
</tr>
<tr>
<td>267 002</td>
<td>200 tests</td>
</tr>
</tbody>
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