DIRECT HDL CHOLESTEROL

For the direct, quantitative measurement of High-Density Lipoprotein Cholesterol (HDL-C) concentration in human serum or plasma.

**PRINCIPLE:**
HDL-L reagent is produced by using a combination of detergents and phosphorous compounds which specifically bind LDL, VLDL and chylomicron (CM) but not HDL. The combination protects LDL, VLDL and CM from the reaction by cholesterol esterase and cholesterol oxidase. Consequently HDL-cholesterol is selectively exposed to react with both enzymes.

\[
\text{HDL (ester-cholesterol) } + \text{H}_2\text{O}_2 \xrightarrow{\text{CE, IP Compounds}} \text{Cholesterol + FattyAcids}
\]

\[
\text{Free Cholesterol + O}_2 \xrightarrow{\text{Peroxidase}} \text{Delta}^4-\text{Cholestenone + H}_2\text{O}_2
\]

\[
2\text{H}_2\text{O}_2 + \text{HDAOS} \xrightarrow{\text{Peroxidase}} 4\text{H}_2\text{O} + \text{Quinone dye} \quad (\lambda_{\text{max} 585 \text{ nm}})
\]

HDAOS = N-(2-hydroxy-3-sulfopropyl)-3, 5 dimethoxyaniline.

**DIAGNOSTIC SIGNIFICANCE:**
High-Density Lipoproteins (HDL) are one of the major classes of plasma lipoproteins. They are composed of a number of heterogeneous particles, including cholesterol and vary with respect to size and content of lipid and apolipoprotein. HDL serves to remove cholesterol from the peripheral cells of the liver, where the cholesterol is converted to bile acids and excreted into the intestine.

An inverse relationship between HDL - Cholesterol (HDL-C) levels in serum and the incidence/prevalence of coronary heart disease (CHD) has been demonstrated in a number of epidemiological studies. The importance of HDL-C as a risk factor for CHD is now recognized.

An accurate measurement of HDL-C is of vital importance when assessing patient risk from CHD. In this diagnostic test, a method for direct measurement of HDL-C, without sample pretreatment is presented. Direct measurement gives improved accuracy and reproducibility when compared to precipitation and ultracentrifugation methods.

**REAGENT COMPOSITION:**
Store all reagents at 2 – 8°C.

<table>
<thead>
<tr>
<th>No. Of Bottles</th>
<th>20 ml/50 T</th>
<th>40 ml/100 T</th>
<th>80 ml/200 T</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

**PRECAUTION:**
For in vitro diagnostic use only. Do not pipette by mouth. Exercise the normal precautions required for handling laboratory reagents.

Calibrator is prepared from human serum. All donors have found negative for Hepatitis B surface antigen and Anti HIV and HCV antibodies. However, this calibrator should be handled with the same care used for the patient samples.

**HDL/LDL Calibrator Preparation & Stability:** Refer the calibrator insert before use.

**REAGENT STORAGE & STABILITY:**

**R1. Enzyme Reagent 1**
The contents are ready to use and stable up to expiration date and must be stored unopened at 2-8°C. Once opened, contents are stable for a month at 2-8°C

**R2. Enzyme Reagent 2**
The contents are ready to use and stable up to expiration date and must be stored unopened at 2-8°C. Once opened, contents are stable for a month at 2-8°C

**SPECIMEN COLLECTION:**
Serum and heparinized plasma are acceptable. EDTA plasma is acceptable, but causes decreased results. Do not freeze the samples. If any sample show precipitation, centrifuge before using.

**NOTE:** - For Spectrophotometer & Fully automated Analyser, select 600nm and for Semi Autoanalyser select Filter 578 nm.

**REACTION PARAMETERS: (A) FOR FIXED-TIME**
- Type of Reaction : Fixed Time
- Wavelength : 600 OR 578 nm
- Flow cell Temperature : 37°C
- Delay Time : 300 Secs
- Interval Time : 320 Secs
- No. of Readings : 2
- Sample Volume : 4 µl
- Reagent Volume (R1 + R2) : 300 + 100 µl
- Calibrator concentration : As mentioned on vial
- Light Path : 1 cm
- Zero Setting with : Distilled Water

**PROCEDURE: (FOR FIX TIME)**

Pipette Into Test tubes

<table>
<thead>
<tr>
<th>Pipette Into</th>
<th>Test tubes</th>
<th>Calibrator</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1 HDL Cholesterol (µl)</td>
<td>300</td>
<td>300</td>
<td></td>
</tr>
<tr>
<td>Calibrator (µl)</td>
<td>4</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>Sample (µl)</td>
<td>---</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>

Mix well and Incubate at 37°C for 5 minutes. Take the initial reading at 600 nm or 578 nm after the incubation. (A1)

| R2 HDL Cholesterol (µl) | 100 | 100 |

Mix well and again incubate at 37°C for 5 minutes and take the final reading at 600 nm or 578 nm (A2). Determine the change in absorbance as per:

\[
\Delta \text{A} = (A2-A1) \text{ Value.}
\]
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REACTION PARAMETERS : (B) FOR END-POINT
- Type of Reaction: End Point
- Wavelength: 600 OR 578 nm
- Flow cell Temperature: 37°C
- Incubation: 5 + 5 min.
- Sample/Calibrator Volume: 10 µl
- Reagent Volume (R1 + R2): 750 + 250 µl
- Calibrator concentration: As mentioned on vial
- Light Path: 1 cm
- Zero Setting with: Reagent Blank

PROCEDURE: (FOR END POINT)

Pipette Into Test tubes
<table>
<thead>
<tr>
<th>Blank</th>
<th>Calibrator</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1 HDL Cholesterol (µl)</td>
<td>750</td>
<td>750</td>
</tr>
<tr>
<td>Calibrator (µl)</td>
<td>-</td>
<td>10</td>
</tr>
<tr>
<td>Sample (µl)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Mix well and Incubate at 37°C for 5 minutes.

R2 HDL Cholesterol (µl) | 250 | 250 | 250 |
Mix well and again incubate at 37°C for 5 minutes. Read the absorbance of test and calibrator at 600 OR 578 nm. against blank.

CALCULATION: (For fix-Time and Endpoint)

\[
\text{HDL-C Concentration (mg/dl)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of Calibrator}} \times \text{Calib. Concentration}
\]

QUALITY CONTROL:
Quality control materials are intended for use only to monitor accuracy and precision. The values for these controls should fall within specified limits. If the control values fall outside these ranges and repetition preclude technical error, the following steps should be taken.
1. Check wavelength setting and light source.
2. Ensure that cuvettes and glassware in use have been thoroughly cleaned.
3. Check water contaminants (e.g. Bacterial Growth) may contribute to inaccurate results.
4. Check that assay temperature is accurate.
5. Ensure that reagent pack contents are still within expiration date.

EXPECTED VALUES:

<table>
<thead>
<tr>
<th>No Risk</th>
<th>Moderate Risk</th>
<th>High Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEN</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mg/dl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;55</td>
<td>35-55</td>
<td>&lt;35</td>
</tr>
<tr>
<td>&lt;1.45</td>
<td>0.90 - 1.45</td>
<td>&lt;0.90</td>
</tr>
<tr>
<td>WOMEN</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mg/dl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;65</td>
<td>45-65</td>
<td>&lt;45</td>
</tr>
<tr>
<td>&gt;1.68</td>
<td>1.15-1.68</td>
<td>&lt;1.15</td>
</tr>
</tbody>
</table>

National Cholesterol Education Program (NCEP) Guidelines

- <35 mg/dl: low HDL Cholesterol (Major risk factor for CHD)
- >60 mg/dl: high HDL Cholesterol (Negative risk factor for CHD)

As HDL Cholesterol is affected by a number of factors such as smoking, exercise, hormones, age and sex, each laboratory should establish its own reference ranges.

INTERFERENCES:
No interference of Ascorbic Acid up to 100 mg/dl
No interference of Bilirubin & Bilirubin Conjugate up to 40 mg/dl
No interference of Hemoglobin up to 500 mg/dl

LINEARITY:
The method is linear up to 220 mg/dl. Samples above this concentration should be diluted 1:1 with 0.9 % (w/v) NaCl and reassayed. The result must then be multiplied by 2.

SPECIFIC PERFORMANCE CHARACTERISTICS:
The following performance characteristics were obtained using Hitachi 917 analyzer. Users should establish their own performance characteristics for their systems.

PRECISION (SERUM)

<table>
<thead>
<tr>
<th></th>
<th>Serum 1</th>
<th>Serum 2</th>
<th>Serum 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>30</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Mean</td>
<td>32.6</td>
<td>47.3</td>
<td>68.5</td>
</tr>
<tr>
<td>Min.</td>
<td>32.2</td>
<td>46.6</td>
<td>67.7</td>
</tr>
<tr>
<td>Max.</td>
<td>33.0</td>
<td>48.2</td>
<td>69.6</td>
</tr>
<tr>
<td>CV (%)</td>
<td>0.65</td>
<td>0.78</td>
<td>0.65</td>
</tr>
</tbody>
</table>

REFERENCES: