ORDER INFORMATION

<table>
<thead>
<tr>
<th>REF:</th>
<th>DLDL 20</th>
<th>Cont.</th>
<th>1x20 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DLDL 40</td>
<td></td>
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</tbody>
</table>

CLINICAL SIGNIFICANCE

The LDL particles are lipoproteins that transport cholesterol to the cells. Often called "bad cholesterol" because high levels are risk factor for coronary heart disease and are associated with obesity, diabetes and nephrosis. Clinical diagnosis should not be made on a single test result; it should integrate with clinical and other laboratory data.

PRINCIPLE

Direct determination of serum LDL (low-density lipoprotein cholesterol) levels without the need for any pre-treatment or centrifugation steps. The assay takes place in two steps:

1. Elimination of lipoprotein non-LDL

   - Cholesterol esters $\rightarrow$ Cholesterol + Fatty acids
   - Cholesterol + O$_2$ $\rightarrow$ 4-Cholestenone + H$_2$O$_2$
   - 2H$_2$O$_2$ $\rightarrow$ 2H$_2$O + O$_2$

2. Measurement of LDLc

   - Cholesterol esters $\rightarrow$ Cholesterol + Fatty acids
   - Cholesterol + O$_2$ $\rightarrow$ 4-Cholestenone + H$_2$O$_2$
   - 2H$_2$O$_2$ + TOOS + 4-AA $\rightarrow$ 2H$_2$O + O$_2$

The intensity of the color formed is proportional to the LDL concentration in the sample.

REAGENT COMPOSITION

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td></td>
</tr>
<tr>
<td>Cholesterol esterase (CHE)</td>
<td>380U/L</td>
</tr>
<tr>
<td>Cholesterol oxidase (CHOD)</td>
<td>380U/L</td>
</tr>
<tr>
<td>Catalase</td>
<td>400 U/mL</td>
</tr>
<tr>
<td>N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline (TOOS)</td>
<td>0.45 mmol/L</td>
</tr>
<tr>
<td>HDLc/LDLc CAL</td>
<td>Standard, Lyophilized human serum</td>
</tr>
</tbody>
</table>

SAFETY PRECAUTIONS AND WARNINGS

HDL/LDL CAL

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: After serum separation, the test should be performed without delay. Repeated freezing and thawing should be avoided.

Stability of the sample: 7 days at 2-8°C

REAGENT PREPARATION AND STORAGE

- R1 and R2: Are ready to use.
- HDL/LDL CAL: Dissolve the contents with 0.5 mL of distilled water. Cap vial and mix gently to dissolve contents.

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

1000 mg/dl

ASSAY PROCEDURE

1. Assay conditions:
   - Wavelength: 600 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>Component</th>
<th>Blank</th>
<th>Standard</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1 (μL)</td>
<td>300</td>
<td>300</td>
<td>300</td>
</tr>
<tr>
<td>Standard (μL)</td>
<td>-</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>Sample (μL)</td>
<td>-</td>
<td>-</td>
<td>4</td>
</tr>
</tbody>
</table>

4. Mix and Incubate for 5 min at 37°C.
5. Add:

   | R2 (μL) | 100   | 100   | 100   |

6. Mix and Incubate for 5 min at 37°C.
7. Read the absorbance (A), against the Blank.
CALCULATION

A Sample

\[ \text{mg/dL of LDLc in the sample} = \text{Calibrator conc.} \times \text{Calibrator} \]

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.

REFERENCE INTERVAL

Levels of the risk
Desirable \(<100 \text{ mg/dL}\)
Medium \(>100 \text{ mg/dL}\)
High \(>160 \text{ mg/dL}\)

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

PERFORMANCE CHARACTERISTICS

Measuring range: From detection limit of 3.7 mg/dL to linearity limit of 1000 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</th>
<th>Inter-assay</th>
</tr>
</thead>
<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>
</tbody>
</table>

Sensitivity: 1mg/dL = 0.0012 A.

Accuracy: Results obtained using ACCUCARE reagents (y) did not show systematic differences when compared with other commercial reagents. (x).
The results obtained using 92 samples were the following.
Correction coefficient \((r) = 0.996\).
Regression equation: \(y = 4.6 + 0.940x\).
The results of the performance characteristics depend on the analyzer used.

INTERFERENCES

No Interferences were observed with ascorbic acid up to 50 mg/dL, hemoglobin up to 500 mg/dL, or bilirubin up to 30 mg/dL. A list of drugs and other interfering substances with LDL cholesterol determination has been reported by Young et al 8,4

NOTES

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

BIBLIOGRAPHY