**LDL Cholesterol Fluid**

**ENZYMATIC IN VITRO ASSAY FOR THE DIRECT QUANTITATIVE DETERMINATION OF LDL-CHOLESTEROL IN HUMAN SERUM AND PLASMA**

**QUALITY CONTROL**

The control intervals and limits must be adapted to the individual laboratory and country-specific requirements. Values obtained should fall within established limits. Each laboratory should establish corrective measures to be taken if values fall outside the limits.

**SUMMARY**

LDL is responsible for the reverse transport of cholesterol from the peripheral cells to the liver. Here, cholesterol is transformed to bile acids which are excreted into the intestine via the biliary tract. Monitoring of LDL-cholesterol in serum is of clinical importance since an inverse correlation exists between serum LDL-cholesterol concentrations and the risk of atherosclerotic disease. Elevated LDL-cholesterol concentrations are protective against coronary heart disease, while reduced HDL-cholesterol concentrations, particularly in conjunction with elevated triglycerides, increase the cardiovascular risk.

A variety of methods are available to determine LDL-cholesterol, including ultracentrifugation, electrophoresis, HPLC, and precipitation-bases methods. Of these precipitation-based methods are used routinely. LDL-cholesterol is first separated by precipitating apoprotein B-containing lipoproteins from serum by using a combination of a polyanion and a divalent cation, such as dextran sulfate/magnesium chloride or phosphotungstate/magnesium chloride.

**TEST PRINCIPLE**

The low density lipoproteins (LDL) are precipitated by heparin at their isoelectric point (pH 5.12). After centrifugation the high density lipoproteins (HDL) and the very low density lipoproteins (VLDL) remain in the supernatant and can then be determined enzymatically. The LDL-cholesterol can be calculated as the difference between supernatant cholesterol and total serum.

**NOTES**

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.

**LIMITATIONS - INTERFERENCE**

The values obtained are reliable, provided that:
- no chylomicrons are present in the sample
- the triglyceride concentration does not exceed 400 mg/dl
- the sample does not show signs of type III hyperlipoproteinaemia

In measurement at Hg 546 nm, the spectral properties of hemoglobin simulate elevated LDL cholesterol values which can be ignored up to 200 mg Hb/100 ml. The supernatant obtained on centrifugation must be clear. If the sample has a high triglyceride content (above 1000mg/dl), lipoprotein precipitation may be incomplete (cloudy supernatant), or part of the precipitate may float on the surface. In these cases, dilute the specimen 1 + 1 with 0.9% NaCl solution and repeat the precipitation step. The result of the cholesterol assay must then be multiplied by 2. High concentrations of ascorbic acid may result in artificially low values.

**Interferences:**

- Criterion: Recovery within 10% of initial value.
- Icterus: No significant interference up to an index I of 20 for bilirubin and (approximate bilirubin conc.: 20 mg/dl)
- Hemolysis: No significant interference up to an index H of 300 (approximate hemoglobin conc.: 300 mg/dl).

**Expected Values**

Values used as criteria for treatment:
- No treatment required <150 mg/dl
- Suspect range 150 – 190 mg/dl
- Treatment required > 190 mg/dl

National Cholesterol Education Program (NCEP) guidelines:
- < 35 mg/dl Low LDL-Cholesterol (major risk factor for CHD)
- > 60 mg/dl High LDL-Cholesterol (negative risk factor for CHD) LDL-Cholesterol is affected by a number of factors, e.g., smoking, exercise, hormones, sex and age.

Each laboratory should investigate the transferability of the expected values to its own patient population and, if necessary, determine its own reference range.

**ANALYTICAL SENSITIVITY**

Detection limit: 3 mg/dl (0.08 mmol/l)

The lower detection limit represents the lowest measurable LDL-cholesterol concentration that can be distinguished from zero.

**IMPRECISION**

Reproducibility was determined using an internal protocol. The following results were obtained.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean mg/dl</th>
<th>SD mg/dl</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>30.13</td>
<td>0.75</td>
<td>2.49</td>
</tr>
<tr>
<td>Sample 2</td>
<td>113.51</td>
<td>0.75</td>
<td>0.66</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean mg/dl</th>
<th>SD mg/dl</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>59.4</td>
<td>2.15</td>
<td>3.61</td>
</tr>
<tr>
<td>Sample 2</td>
<td>84.2</td>
<td>2.64</td>
<td>3.14</td>
</tr>
</tbody>
</table>

**METHOD COMPARISON**

A comparison of the AXIOM LDL Cholesterol Fluid (y) with a commercial obtainable assay (x) gave the following result (mg/dl):

\[ y = 0.956x + 8.049 ; r = 0.956 \]

**REAGENT CONCENTRATION**

- R1
  - Heparin: 0.68 g/l
  - Sodium citrate: 0.064 mol/l
  - Stabilizers: 2%

**PREPARATION AND STABILITY**

Precipitant for macro assays. Use contents undiluted.

The LDL reagent is stable up to the expiry date when stored at +15°C to +25°C.

**SPECIMEN**

Collect serum using standard sampling tubes
- Li-heparin and Na-heparin - plasma
Do not use citrate-, oxalate- or fluoride-plasma!

Stability:
- 7 days at +2°C to +4°C
- 3 months at -20°C

Fasting and non-fasting samples can be used. EDTA plasma causes decreased results.

Centrifuge samples containing precipitate before performing the assay.

**Testing Procedure**

**Materials provided**
- Working solutions as described above
- Additional materials required:
  - 0.9% NaCl
  - Cholesterol standard (100 mg/dL)
  - LDL reagent

**Additional requirements**
- Centrifuge samples containing precipitate before performing the assay.

**Precipitation**

Pipette into centrifuge tubes:
- Standard/Sample: 100 µl
- LDL reagent: 1000 µl

Mix well, let stand for 10 minutes at +15°C to +25°C and centrifuge for 10 minutes at 10,000 rpm. After centrifugation separate the clear supernatant from the precipitate within 1 hour and determine the cholesterol concentration.

**Cholesterol Determination**

<table>
<thead>
<tr>
<th>测定</th>
<th>Blank</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biodest. H₂O</td>
<td>100 µl</td>
<td>100 µl</td>
</tr>
<tr>
<td>Supernatant</td>
<td>100 µl</td>
<td>100 µl</td>
</tr>
<tr>
<td>Cholesterol reagent</td>
<td>1000 µl</td>
<td>1000 µl</td>
</tr>
</tbody>
</table>

Mix, measure after incubating at +37°C for 5 minutes or 10 minutes at 20°C to +25°C. Determine ΔA/min.

**Calculation by factor**

Hg 546 nm

$\text{mg/dL} = \frac{1000 \times \Delta A}{\text{mg/dL}}$

**Calculation by standard**

<table>
<thead>
<tr>
<th>Standard conc. (mg/dl)</th>
<th>Concentration of LDL-cholesterol in the supernatant (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 µl</td>
<td>Conc. of LDL-Cholesterol = Conc. of Total Cholesterol - Conc. of Cholesterol in the supernatant.</td>
</tr>
</tbody>
</table>

**Disposal**

Please note the legal regulations.

**Literature**

1. Assmann G, Schuler H, Schmitz G et al. Quantification of high density lipoprotein cholesterol by precipitation with phosphotungstic acid/MgCl₂ Clin Chem 1983;29:2026-2030

**Axiom Product range Clinical Chemistry**

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Ions</th>
<th>Other Metabolites</th>
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</thead>
<tbody>
<tr>
<td>Acid Phosphatase</td>
<td>Ammonium fluid</td>
<td>Bilirubin T/D</td>
</tr>
<tr>
<td>Alkaline Phosphatase</td>
<td>Copper fluid</td>
<td>Creatinine fluid</td>
</tr>
<tr>
<td>$\alpha$-Amylase</td>
<td>Calcium fluid</td>
<td>Glucose GOD-PAP fluid</td>
</tr>
<tr>
<td>CK-NAC</td>
<td>Chloride fluid</td>
<td>Glucose Hexokinase fluid</td>
</tr>
<tr>
<td>CK-MB (NAC-activated)</td>
<td>Inorganic Phosphorus UV fluid</td>
<td>Urea Enzymatic fluid</td>
</tr>
<tr>
<td>$\gamma$-GT</td>
<td>Iron fluid</td>
<td>Urea UV fluid</td>
</tr>
<tr>
<td>LDH</td>
<td>TIBC</td>
<td>Uric Acid PAP fluid</td>
</tr>
<tr>
<td>Cholestesterase</td>
<td>Magnesium fluid</td>
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<tr>
<td>GOT/ASAT</td>
<td>Potassium fluid</td>
<td>Controls</td>
</tr>
<tr>
<td>GPT/ALT</td>
<td>Sodium fluid</td>
<td>Control Serum N</td>
</tr>
<tr>
<td>Lactate PAP</td>
<td></td>
<td>Control serum P</td>
</tr>
<tr>
<td>$\alpha$-HBDH</td>
<td></td>
<td>Protein</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Lids</th>
<th>Albumin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol fluid</td>
<td>Microprotein fluid</td>
</tr>
<tr>
<td>HDL Cholesterol</td>
<td>Hemoglobin</td>
</tr>
<tr>
<td>LDL Cholesterol</td>
<td>Protein Total fluid</td>
</tr>
</tbody>
</table>