Mr.CHOLESTEROL

DIAGNOSTIC KIT FOR DETERMINATION OF TOTAL CHOLESTEROL CONCENTRATION

Kit name          Kit size          Cat. No
Mr.Cholesterol mini 2 x 25 ml        GB11MR
Mr.Cholesterol 100 2 x 50 ml        GB12MR
Mr.Cholesterol 200 4 x 50 ml        GB13MR
Mr.Cholesterol 500 5 x 100 ml       GB01BK

INTRODUCTION - Total Cholesterol
Cholesterol is essential structural component of cell membranes and precursor of bile acids and all steroids hormones. This is why cholesterol has enormous significance for organism normal functioning. But there is also well established association between blood cholesterol concentration and coronary heart disease. Measurement of cholesterol serum level is valuable in prevention and monitoring cardiovascular disease. This determination is useful also for evaluation of intestine absorption, liver and gallbladder function.

INTRODUCTION - HDL Cholesterol
High Density Lipoproteins (HDL) contain particles of different density including lipid and highest concentration of proteins amongst the different lipoproteins. It includes free and esterified cholesterol, triglycerides, phospholipids and apoproteins A, C and E. HDL cholesterol values are about 1/5th of the total cholesterol values and can be expressed as percentage of total cholesterol. There exists an increase relationship between HDL cholesterol and coronary heart diseases. Low concentration i.e., below 30 mg/dl is one of the risk factors for cardiac ailments.

METHOD PRINCIPLE - Total Cholesterol
Enzymatic, colorimetric method with cholesterol esterase and cholesterol oxidase (CHOD/PAP).

\[
\text{cholesterol esters} + \text{H}_2\text{O} \rightarrow \text{cholesterol} + \text{fatty acids}
\]

\[
\text{Cholesterol} + \text{O}_2 + 4\text{-aminoantipyrine} + \text{phenol} + \text{quinonemine dye} + 4\text{H}_2\text{O} \rightarrow \text{Red coloured}
\]

The colour intensity is proportional to the cholesterol concentration.

METHOD PRINCIPLE - HDL Cholesterol
Chylomicrons, LDL and VLDL (low and very low density lipoproteins) are precipitated from serum by phosphotungstate in the presence of divalent cations such as magnesium. The HDL cholesterol remains unaffected in the supernatent and is estimated using Total Cholesterol reagent.

Serum/plasma Phosphotungstate HDL + (LDL + VLDL)

REAGENTS Package

Mr.CHOL Mini Mr.CHOL 100 Mr.CHOL 200 Mr.CHOL 500
R1-CHOL 2 x 25 ml 2 x 50 ml 4 x 50 ml 5 x 100 ml
R2-STANDARD 1 vial 1 vial 1 vial 1 vial
R3-HDL ppt rgt 1 x 10 ml 1 x 10 ml 1 x 10 ml 1 x 10 ml
R4-STANDARD 1 vial 1 vial 1 vial 1 vial

R2-STANDARD is cholesterol standard solution: Refer Standard Vial
R4-STANDARD is HDL cholesterol standard solution: Refer standard vial

Working reagent preparation and stability
The reagent is ready to use. The reagent when stored at 2-8°C is stable up to expiry date printed on the package. The reagents are stable for 8 weeks on board the analyser at 2-10°C. Protect from light and avoid contamination.

Concentrations in the test

<table>
<thead>
<tr>
<th>Standard</th>
<th>Test</th>
<th>Reagent blank</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(RB)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(T)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(S)</td>
</tr>
<tr>
<td>Good's buffer (pH 6.4)</td>
<td>100 mmol/l</td>
<td>5 mmol/l</td>
</tr>
<tr>
<td>phenol</td>
<td>0.3 mmol/l</td>
<td></td>
</tr>
<tr>
<td>4-aminoantipyrine</td>
<td>&gt; 3.2 µkat/l</td>
<td></td>
</tr>
<tr>
<td>cholesterol esterase (CHE)</td>
<td>&gt; 1.67 µkat/l</td>
<td></td>
</tr>
<tr>
<td>cholesterol oxidase (CHO)</td>
<td>&gt; 3.2 µkat/l</td>
<td></td>
</tr>
<tr>
<td>peroxidase (POD)</td>
<td>&gt; 50 µkat/l</td>
<td></td>
</tr>
<tr>
<td>Phosphotungstic Acid</td>
<td>2.4 mmol/l</td>
<td></td>
</tr>
<tr>
<td>Magnesium Chloride</td>
<td>25 mg/dl</td>
<td></td>
</tr>
</tbody>
</table>

WARNINGS AND NOTES

Product for in vitro diagnostic use only.

The reagents are usable when the absorbance of the working reagent is less than 0.150 (read against distilled water, wavelength \(\lambda\) = 500 nm, cuvette l = 1 cm, at temp. 25°C).

The reagent and standards contain 0.09% sodium azide as a preservative. Avoid contact with skin and mucous membranes.

ADDITIONAL EQUIPMENT

- automatic analyzer or photometer able to read at 500 nm (Hg 546 nm)
- thermostat at 37°C
- General laboratory equipment

SPECIMEN

Serum, EDTA or heparinized plasma (recommended: heparine lithium, sodium or ammonium salt) free from hemolysis. Blood should be collected only if the patient has been fasting for minimum of 12 hours. Before blood collection patient should stay in rest position for about 30 minutes. Venous blood is recommended for cholesterol measurement.

Plasma cholesterol values have been reported to be 3% to 5% lower than serum cholesterol values.

Serum should be separated from red blood cells as soon as possible after blood collection.

Serum and plasma can be stored up to 3 days at 2-8°C or 6 months at -20°C. Nevertheless it is recommended to perform the assay with freshly collected samples.

PROCEDURE FOR TOTAL CHOLESTEROL

These reagents may be used both for manual assay and in several automatic analysers. Applications for them are available on request.

Manual procedure

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<td></td>
<td></td>
<td>(T)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(S)</td>
</tr>
<tr>
<td>wavelength</td>
<td>500 nm (Hg 546 nm)</td>
<td></td>
</tr>
<tr>
<td>temperature</td>
<td>20-25°C / 37°C</td>
<td></td>
</tr>
<tr>
<td>cuvette</td>
<td>1 cm</td>
<td></td>
</tr>
</tbody>
</table>

Pipette into the cuvettes:

Mix well, incubate for 5 min. at 37°C or 10 min. at 20-25°C. Read the absorbance of the test (T) and standard (S) against reagent blank (RB).

Calculation

cholesterol concentration = \(\frac{A(T)}{A(S)}\) x standard concentration
Calculation

\[
\text{HDL Cholesterol} = \frac{A(T) \times \text{concentration of HDL standard}}{A(S)} \times 50 \times 2
\]

LINEARITY: upto 125 mg/dl or 3 mmol/l of HDL.

WASTE MANAGEMENT

Please refer to local legal requirements.

LITERATURE


SYSTEM PARAMETERS

- Method : Endpoint
- Wavelength : 505 nm
- Zero Setting : Reagent Blank
- Temperature Setting : 37°C
- Incubation Temperature : 37°C
- Incubation Time : 5 mins
- Delay time : --
- Read time : --
- No. of Reading : --
- Interval time : --
- Sample Volume : 0.01 ml (10 ul)
- Reagent Volume : 1.0 ml (1000 ul)
- Standard Concentration : Refer Standard Vial
- Units : mg/dl
- Factor : --
- Reaction slope : Increasing
- Linearity : 750 mg/dl

For HDL Cholesterol

Sample Volume : 0.05 ml (50 ul)
Reagent Volume : 1.0 ml (1000 ul)
Standard Concentration : 50 mg/dl x 2 (dilution factor)

It is recommended for each laboratory to establish its own reference ranges for local population.

QUALITY CONTROL

To ensure adequate quality control, each run should include assayed normal and abnormal controls. If commercial controls are not available it is recommended that known value samples be aliquoted, frozen and used as controls.

REFERENCE VALUES

<table>
<thead>
<tr>
<th>Serum / plasma</th>
<th>mg/dl</th>
<th>mmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>children ≤ 4 wk</td>
<td>50-170</td>
<td>1.3-4.4</td>
</tr>
<tr>
<td>2 – 12 mo</td>
<td>60-190</td>
<td>1.6-4.9</td>
</tr>
<tr>
<td>≥ 1 y</td>
<td>110-230</td>
<td>2.8-6.0</td>
</tr>
<tr>
<td>adults</td>
<td>&lt; 200</td>
<td>&lt; 5.2</td>
</tr>
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