**INTENDED USE**
For the quantitative *in vitro* determination of HDL-cholesterol in serum and plasma. This product is suitable for Manual use.

Supplementary pack for Cholesterol, CHOD-PAP method.

**Cat. No.**
CH 203 R1. Precipitant 4 x 80 ml

**CLINICAL 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 serve to remove cholesterol from the peripheral cells to 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 recognised.

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

**PRINCIPLE**
Low density lipoproteins (LDL and VLDL) and chylomicron fractions are precipitated quantitatively by the addition of phosphotungstic acid in the presence of magnesium ions. After centrifugation, the cholesterol concentration in the HDL (high density lipoprotein) fraction, which remains in the supernatant, is determined.

**SAMPLE**
Serum, heparinized plasma or EDTA plasma.

**REAGENT COMPOSITION**

<table>
<thead>
<tr>
<th>Contents</th>
<th>Initial Concentrations of Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1. Phosphotungstic Acid</td>
<td>0.55 mmol/l</td>
</tr>
<tr>
<td>Magnesium Chloride</td>
<td>25 mmol/l</td>
</tr>
</tbody>
</table>

**SAFETY PRECAUTIONS AND WARNINGS**
*For in vitro diagnostic use only. Do not pipette by mouth. Exercise the normal precautions required for handling laboratory reagents.*

Health and Safety Data Sheets are available on request.

The reagents must be used only for the purpose intended by suitably qualified laboratory personnel, under appropriate laboratory conditions.

**STABILITY AND PREPARATION OF REAGENTS**

**R1. Macro assays:** Contents ready for use undiluted. Stable up to the expiry date specified when stored at +15 to +25°C.

**R1. Semi-micro assays:** Predilute the precipitating reagent in the ratio 4 + 1 with redistilled water (dilute the contents of 80 ml bottle with 20 ml redistilled water). Stable up to the expiry date specified when stored at +15 to +25°C.

**MATERIALS PROVIDED**
HDL-Cholesterol Precipitant

**MATERIALS REQUIRED BUT NOT PROVIDED**
Randox Lipid Controls:-
Level 1  LE 2661 or LE 2668
Level 2  LE 2662 or LE 2669
Level 3  LE 2663 or LE 2670
Randox Aqueous Cholesterol Standard Cat. No. ST 1590

**PROCEDURE NOTES**
Only clear supernatants on centrifugation must be used.
In the case of incomplete sedimentation (turbid supernatant) caused by elevated triglyceride concentrations, the sample should be diluted 1 + 1 with 0.9% NaCl solution and the precipitating step repeated. The result should then be multiplied by 2.

LDL Cholesterol: The values obtained using this assay are reliable provided that no chylomicrons are present in the sample, the triglyceride concentration does not exceed 400 mg/dl, and the sample does not show signs of any type III hyperlipoproteinaemia.

**PROCEDURE**

1. **Precipitation**

   Pipette into centrifuge tubes:

<table>
<thead>
<tr>
<th>Macro</th>
<th>Semi Micro</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample/Standard 500 µl 200 µl</td>
<td></td>
</tr>
<tr>
<td>Precipitant (R1) 1000 µl --</td>
<td></td>
</tr>
<tr>
<td>Diluted Precipitant (R1) --- 500 µl</td>
<td></td>
</tr>
</tbody>
</table>

   Mix and allow to sit for 10 minutes at room temperature. Then centrifuge for 10 minutes at 4,000 rpm, or 2 minutes at 12,000 rpm.

   Separate off the clear supernatant within two hours and determine the cholesterol content by the CHOD-PAP method. The supernatant may be stored up to five days at +2 to +25°C.
2. Cholesterol CHOD-PAP Assay

Wavelength: 500 nm, Hg 546 nm
Cuvette: 1 cm light path
Temperature: 20-25°C or 37°C
Measurement: against reagent blank

Only one reagent blank per series is required.

Pipette into test tubes:

<table>
<thead>
<tr>
<th></th>
<th>Reagent Blank</th>
<th>Standard</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled Water</td>
<td>100 µl</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Supernatant</td>
<td>--</td>
<td>--</td>
<td>100 µl</td>
</tr>
<tr>
<td>Standard Supernatant</td>
<td>--</td>
<td>100 µl</td>
<td>--</td>
</tr>
<tr>
<td>Reagent</td>
<td>1000 µl</td>
<td>1000 µl</td>
<td>1000 µl</td>
</tr>
</tbody>
</table>

Mix, incubate for 10 minutes at 20-25°C or 5 minutes at 37°C. Measure the absorbance of the sample (Asample) and standard (Astandard) against the reagent blank within 60 minutes.

**CALCULATION**

1. **HDL Cholesterol**

When using a factor:

<table>
<thead>
<tr>
<th></th>
<th>MACRO</th>
<th>SEMI-MICRO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength</td>
<td>mmol/l</td>
<td>mg/dl</td>
</tr>
<tr>
<td>500 nm</td>
<td>4.65</td>
<td>180</td>
</tr>
<tr>
<td>Hg 546 nm</td>
<td>7.09</td>
<td>274</td>
</tr>
</tbody>
</table>

When using a standard:

Concentration of HDL Cholesterol in supernatant.

\[
\text{Conc. of Standard} \times \frac{\Delta A_{\text{Sample}}}{\Delta A_{\text{Standard}}} = \text{Conc. of HDL Cholesterol}
\]

2. **LDL Cholesterol**

In mmol/l:

\[
\text{LDL Cholesterol} = \text{Total Cholesterol} - \frac{\text{Triglycerides}}{2.2} - \text{HDL Cholesterol}
\]

In mg/dl:

\[
\text{LDL Cholesterol} = \text{Total Cholesterol} - \frac{\text{Triglycerides}}{5} - \text{HDL Cholesterol}
\]

**QUALITY CONTROL**

Randox Lipid Controls, Level 1, Level 2 and Level 3 are recommended for daily quality control. Two levels of controls should be assayed at least once a day. Values obtained should fall within a specified range. If these values fall outside the range and repetition excludes error, the following steps should be taken:

1. Check instrument settings and light source.
2. Check cleanliness of all equipment in use.
3. Check water, contaminants i.e. bacterial growth may contribute to inaccurate results.
4. Check reaction temperature.
5. Check expiry date of kit and contents.
6. Contact Randox Laboratories Customer Technical Support, Northern Ireland (028) 94422413.

**INTERFERENCES**

The assay is unaffected by icteric samples (bilirubin < 30 mg/dl), rheumatoid factors <1000 IU/ml, haemolytic samples (Hb < 500 mg/dl) and lipaemic samples (triglyceride < 1200 mg/dl). Lipaemic samples with a triglyceride concentration >1200 mg/dl should be diluted 1+9 with 0.9% (w/v) NaCl before assay. The corresponding result should be multiplied by 10.

**EXPECTED VALUES (4,5) (NCEP GUIDELINES)**

<table>
<thead>
<tr>
<th></th>
<th>mg/dl</th>
<th>mmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 40</td>
<td>&lt;1.04</td>
<td>Low</td>
</tr>
<tr>
<td>≥60</td>
<td>≥1.55</td>
<td>High</td>
</tr>
</tbody>
</table>

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.

**LINEARITY**

The test is linear up to a cholesterol concentration of 19.3 mmol/l (750 mg/dl). Dilute samples with a cholesterol concentration greater than this 1+2 with 0.9% NaCl. Multiply the result by 3.

**SENSITIVITY**

It is recommended that each laboratory establishes its own range of sensitivity as this is limited by the sensitivity of the spectrometer used.

Under the conditions of the assay a change of 0.004 absorbance units is equivalent to 0.08 mmol/l.

**REFERENCES**


Revised 30 Jul 07 aw