Cholesterol LiquiColor® Test Procedure No. AL225
Quantitative-Enzymatic-Colorimetric Determination of Total and HDL Cholesterol in Serum or Plasma

Materials Required But Not Provided
Spectrophotometer capable of absorbance readings at 500 nm.
Accurate pipetting devices
Cuvets Test Tubes Centrifuge Interval Timer Mixerc (Vortex type)
Constant temperature bath, or block, 37°C (Optimal)

Specimen Collection and Preparation
Blood should be collected following a 12-hour fast. Specimen may be serum, or plasma collected with EDTA as anticoagulant.
Avoid hemolysis.
Sample Stability: Both total cholesterol and HDL cholesterol are reportedly stable 4 days at 2-8°C. Extended stabilities at -20°C are 3 months for “Total” and 7-14 days for “HDL.” Whenever possible, specimens should be separated and analyzed on the day of collection.

Interfering Substances: Anticoagulants such as fluoride and oxalate will result in false low values. The test is not influenced by hemoglobin values up to 200 mg/dL or by bilirubin levels up to 10 mg/dL. However, interference from grossly icteric and heavily hemolyzed specimens is corrigible by use of a serum/plasma blank (refer to “Results” section).

Automated Analysers

- Parameters:
  - Wavelength: 500 nm
  - Reaction Type: Endpoint
  - Reaction Direction: Increasing
  - Reaction Temperature: 37°C
  - Sample/Reagent Ratio: 1:100
  - Equilibration Time: 3 Seconds
  - Read Time: 4 Seconds
  - Lag Time: 300 Seconds
  - Blank Absorbance Limit: 0.30A
  - High Absorbance: 2.00A
  - Standard: 200 mg/dL
  - Low Normal: 120 mg/dL
  - High Normal: 310 mg/dL
  - Linearity: 750mg/dL

Above parameters should be employed in programming automated analyzers for Cholesterol. Consult your instrument manual for specific applications for programming instructions. Specific programming applications for automated analyzers are available from Interchim.

Test procedure

1. Pipet into cuvets the following volumes (mL) and mix well:

<table>
<thead>
<tr>
<th>Reagent/Blank (RB)</th>
<th>Standard (S)</th>
<th>Sample (U)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Standard</td>
<td>—</td>
<td>0.01</td>
</tr>
<tr>
<td>Sample</td>
<td>—</td>
<td>0.01</td>
</tr>
</tbody>
</table>

2. Incubate all cuvets at 37°C for 5 minutes or at room temperature for 10 minutes.

3. Read S and U vs. RB at 500 nm within 60 minutes.

Quality Control: Use of commercial control serum, or pooled serum previously assayed and divided into frozen aliquots, is recommended for use with each series of assays.

Results
Values are derived by the following equations:

Serum Total Cholesterol (mg/dL) = \( \frac{Au - As}{200} \) As

Where Au and As are the absorbance values of unknown and standard, respectively, and 200 the concentration of the standard (mg/dL).

When a serum blank is required (icteric or hemolyzed specimen), label another tube SB (Step 1, “Procedure” section). Add 1.0 mL “normal” saline 0.01 mL serum, mix by inversion, transfer to cuvet and read absorbance (Asb) vs. distilled water at 500 nm. Use this value to correct that of the unknown as follows:

Serum Total Cholesterol (mg/dL) = \( \frac{Au - Asb \times 200}{As} \)

NOTE: Samples having cholesterol values greater than 750 mg/dL are diluted 3-fold (1+2) with normal saline (sodium chloride, 8.5 g/L), the assay repeated and results multiplied by the dilution factor of 3.

Expected Values
Recent data are presented showing “normal ranges” according to age for total and HDL Cholesterol, and as to the risk of coronary heart disease (CHD) for HDL Cholesterol expressed as percent of total cholesterol. It is recommended that each laboratory establish its own range of expected values, since differences exist between instruments, laboratories, and local populations.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Total Cholesterol (mg/dL)</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDL Cholesterol Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;14</td>
<td>30-65</td>
<td>30-70</td>
<td></td>
</tr>
<tr>
<td>15-19</td>
<td>30-65</td>
<td>30-70</td>
<td></td>
</tr>
<tr>
<td>20-29</td>
<td>30-70</td>
<td>30-80</td>
<td></td>
</tr>
<tr>
<td>30-39</td>
<td>30-70</td>
<td>30-85</td>
<td></td>
</tr>
<tr>
<td>&gt;40</td>
<td>30-70</td>
<td>30-85</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>45</td>
<td>56</td>
<td></td>
</tr>
</tbody>
</table>

Blacks: Approx. 10 mg/dL higher

Performance Characteristics
Reproducibility: A study was performed on a control serum (mean = 128 mg/dL) and on a patient’s serum (mean = 367 mg/dL), which consisted of a series of 5 assays on each of 5 days. Coefficients of variation (CV) were within run 2.5% and 1.0% and betweenrun (day-to-day) 3.5% and 2.9%, respectively.

Correlation: Determination of cholesterol by the procedure described (y) and by the Boeringer-Manheim “BMC Cholesterol Monotest” (x) on 66 sera (range 125-550 mg/dL) showed a correlation coefficient (r) of 0.991 and a regression equation of y = 1.04x - 9.3.

Linearity: Linear from 0 to 750 mg/dL.

References

For any information (technical service call, ordering...), please contact Interchim 213 av. J.F. Kennedy, BP 1140 – 03 103 Montlucon (France) Phone: +33 4 70 03 73 06 (hot line) ; Fax: +33 4 70 03 82 60 e-mail: interbiotech@interchim.com; web : http://www.interchim.com

Precautions
For In Vitro Diagnostic Use Only.