Cholesterol determinations are used in characterizing an individual’s risk of developing atherosclerotic disease and aid in the diagnosis and treatment of disorders involving elevated cholesterol levels as well as lipid and lipoprotein metabolic disorders. The determination of triglycerides is utilized in the diagnosis and treatment of patients having diabetes mellitus, metabolic syndrome, lipid metabolism disorders, liver obstruction, and numerous other endocrine diseases.

Note
Please note that the catalogue number appearing on the package insert retains only the first 8 digits of the licensed 11-digit catalogue number, 06380115190 for the cobas Lipid Panel. The last 3 digits -190 have been replaced by -119 for logistic purposes.

Summary
Cholesterol determinations are used in characterizing an individual’s risk of developing atherosclerotic disease and aid in the diagnosis and treatment of disorders involving elevated cholesterol levels as well as lipid and lipoprotein metabolic disorders. Determination of HDL-cholesterol is of clinical importance since an inverse correlation exists between HDL-cholesterol concentrations and the risk of atherosclerotic disease. Elevated HDL-cholesterol concentrations reduce the risk for coronary heart disease, while reduced HDL-cholesterol concentrations, particularly in conjunction with elevated triglycerides, increase the cardiovascular risk. Strategies have emerged to increase the level of HDL-cholesterol to treat cardiovascular disease. The determination of triglycerides is utilized in the diagnosis and treatment of patients having diabetes mellitus, metabolic syndrome, lipid metabolism disorders, liver obstruction, and numerous other endocrine diseases. The determination of a full lipid panel and the calculation of the LDL-cholesterol concentration according to Friedewald’s formula is commonly practiced. Low Density Lipoproteins (LDL) play a key role in causing and influencing the progression of atherosclerosis, and in particular, coronary artery disease. The majority of cholesterol stored in atherosclerotic plaques originate from LDL. The LDL-cholesterol value is the most powerful clinical predictor among all of the single parameters with respect to coronary atherosclerosis. Therefore, therapies focusing on lipid reduction primarily target the reduction of LDL-cholesterol.

Test principle
The erythrocytes of the capillary or venous blood sample are separated from the plasma by centrifugation. In the next step, the plasma sample is diluted with phosphate buffer. The HDL test uses a precipitation method with Mg2+ and phosphotungstic acid as a precipitant reagent. The components except for HDL-cholesterol are precipitated and removed. The cobas b 101 system determines total cholesterol and HDL-cholesterol by an enzymatic method. Cholesterol esters in the sample are hydrolyzed to cholesterol and fatty acids. Cholesterol and NAD+ generate cholesterolone and NADH in the presence of cholesterol dehydrogenase. WST8 is reduced to formazan dye by diaphorase and NADH through oxidation-reduction reaction. The color intensity of formazan is proportional to triglyceride concentration and calculated by measuring at a wavelength of 460 nm.

Through oxidation-reduction reaction. The color intensity of the formazan dye is proportional to triglyceride concentration and calculated by measuring at a wavelength of 460 nm.

Low density lipoprotein (calculated)
Where the concentration of triglycerides is < 400 mg/dL (4.52 mmol/L), the LDL-cholesterol is calculated using the Friedewald formula.

LDL = TC - HDL - TG/5 (measured in mg/dL). Where the concentration of triglycerides is ≥ 400 mg/dL (4.52 mmol/L), the calculated LDL-cholesterol is not reported. The formula is also not valid for non-fasting patients and patients with Type III hyperlipoproteinemia (dysbetalipoproteinemia).

Total Cholesterol/HDL ratio and Non-high density lipoprotein
The cobas b 101 instrument calculates the TC/HDL ratio as well as the non-high density lipoprotein (TC-HDL) from the measured values. Where the measured values data are not available, the TC/HDL ratio or non-HDL-cholesterol values are not reported.

Reagents
One test contains:
- Dilution buffer: potassium dihydrogenphosphate 57 µg, dipotassium hydrogenphosphate 0.3 mg, potassium chloride 2.2 mg, sodium azide 42 µg (≤ 0.02 %)
- Precipitant: magnesium sulfate heptahydrate 48 µg, sodium phosphotungstate n-hydrate 24 µg
- Lipoprotein-lipase 0.096 U, cholesterol esterase 0.5 U, diaphorase 0.77 U, nicotinamide adenine dinucleotide 51 µg, tetrazolium salt 38 µg, glycerol dehydrogenase 0.75 U, cholesterol dehydrogenase 0.84 U

Precautions and warnings
For in vitro diagnostic use. Exercise the normal precautions required for handling all laboratory reagents. Disposal of all waste material should be in accordance with local guidelines. Safety data sheet available for professional user on request.

Handling
Carefully tear open the foil pouch at the tear notch until one side is open. Discard the disc if the foil pouch is found open or damaged, or if the disc is damaged, or the dessicant is missing, or loose dessicant particles or any other dirt or particles especially at the blood application zone are found. Use cobas Lipid Control in the same way as a blood sample.

Storage and stability
Store at 2-30 °C until the expiration date printed on the pouch. Do not freeze. If stored in a refrigerator, allow the test to warm up in the closed pouch for at least 20 minutes before use. Once the pouch is opened, use the test within 20 minutes. Protect the disc from direct sunlight. Do not store opened pouches in a refrigerator. Note: Open the pouch just before use when using control materials.

Specimen collection and preparation
For specimen collection and preparation only use suitable tubes or collection containers.

Use fresh capillary blood. K2 or K3-EDTA venous whole blood or plasma. Do not use other anticoagulants or other additives. Do not freeze samples. We recommend to use EDTA samples within 2 hours to comply with the NCEP goal of bias < 3 % for total cholesterol and bias < 5 % for high density lipoprotein cholesterol. Assure that the lanced site is clean and dry, and free from fatty substances. The marking on the disc clearly shows where to apply the sample. If samples are used from a venipuncture or control material, use a standard pipette or dropper to form a drop. The disc is self-filling. Do not push the sample into the disc. Do not use syringes. Assure that the disc is free from blood outside the sample application zone and the hinge cover.

Sample volume: 19 µL

Sample stability on disc
After applying blood to the disc, insert it into the instrument within 8 minutes. Please follow instructions in the operator’s manual.
Materials provided
REF 06380115190, cobas Lipid Panel, 10 tests

Materials required (but not provided)
- Single use disposable lanceting device (e.g. Accu-Chek Safe-T-Pro Plus)
- REF 06380182190, cobas Lipid Control
- REF 06378688190, cobas b 101 instrument
- Optical check disc
- General laboratory equipment (e.g., sample transfer pipette for venous blood or alcohol wipes for the fingerstick)
- Timer

Calibration
Traceability: Total cholesterol and HDL-cholesterol are traceable to the designated CDC reference methods (Abell/Kendall as reference method for total cholesterol). Triglycerides are traceable to the ID/MS method.

The instrument automatically reads in the lot-specific calibration data from the barcode information printed on the disc, eliminating the need for calibration by the user.

Quality control
For quality control, use cobas Lipid Control.

The control intervals and limits should be adapted to each laboratory’s individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Follow the applicable government regulations and local guidelines for quality control.

QC info disc
Every cobas Lipid Panel contains a lot-specific QC info disc for quality control. This QC info disc contains the target values and ranges for the cobas Lipid Panel.

The instrument display prompts the user to insert the QC info disc. The cobas b 101 instrument reads the disc providing the lot specific target ranges.

Display of results
At the end of the automatic determination, the cobas b 101 instrument displays the result in approximately 6 minutes. The measured total cholesterol, HDL-cholesterol, triglycerides and calculated LDL-cholesterol result will be displayed in mg/dL or mmol/L depending on the setting. Please refer to the operator’s manual.

Limitations - interference
Drugs: No interference was found at therapeutic concentrations using common drug panels. Ascorbic acid: no significant interference up to 5 mg/dL.

Hematocrit levels between 30 % and 55 % do not affect results.

Total cholesterol
Icterus: No significant interference up to 15 mg/dL for conjugated bilirubin and up to 30 mg/dL for unconjugated bilirubin. Hemolysis: No significant interference up to a hemoglobin concentration of 200 mg/dL. Ascorbic acid: no significant interference up to 5 mg/dL. Lipemia (Intralipid): No significant interference up to 500 mg/dL. Criterion: Recovery ≤10 % of initial value at a HDL concentration of 50 mg/dL (1.3 mmol/L).

Abnormal liver function affects lipid metabolism; consequently, HDL-cholesterol and LDL-cholesterol results are of limited diagnostic value. In some patients with abnormal liver function, the HDL-cholesterol result may significantly differ from the designated comparison method (DCM) result.

For diagnostic purposes, the results should always be assessed in conjunction with the patient’s medical history, clinical examination and other findings.

Measuring range
Cholesterol: 50-500 mg/dL or 1.28-12.95 mmol/L
Triglycerides: 45-650 mg/dL or 0.50-7.35 mmol/L
HDL-cholesterol: 15-100 mg/dL or 0.38-2.60 mmol/L

Expected values
The European Society of Cardiology (ESC) and the European Atherosclerosis Society (EAS) jointly published 2011 ESC/EAS guidelines for the management of dyslipidemia. The new guideline recommends the assessment of total cardiovascular (CV) risk. Those with known cardiovascular disease (CVD), type 2 diabetes or type 1 diabetes with microalbuminuria, very high levels of individual risk factors, chronic kidney disease (CKD) are at high or very high total CV risk and need active management of all risk factors. For all other patient groups, the use of a risk estimation system such as SCORE is recommended to estimate total CV risk because many people have several risk factors which, in combination, may result in unexpectedly high levels of total CV risk. Recommendations for lipid analyses for characterization of dyslipidemia before treatment: TC and LDL-C are recommended to be used for the primary lipid analysis for risk estimation of total CV risk. Recommendations for lipid analyses for characterization of dyslipidemia before treatment: TC and LDL-C are recommended to be used for the primary lipid analysis for risk estimation of total CV risk. Recommendations for lipid analyses for characterization of dyslipidemia before treatment: TC and LDL-C are recommended to be used for the primary lipid analysis for risk estimation of total CV risk.

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

Recommendations for lipid analyses as treatment target in the prevention of CVD
Concluding from the available data, an absolute reduction to an LDL-C level < 70 mg/dL (< 1.8 mmol/L) or at least 50 % relative reduction in LDL-C provides the best benefits in terms of CVD reduction. Clinical judgement is required before a final treatment plan is implemented.

National Cholesterol Education Program (NCEP) guidelines
The Third Report of the Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III, or ATP III) presents the National Cholesterol Education Program’s (NCEP’s) updated recommendations for cholesterol testing and management. The ATP III classifications are as follows:

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Concentration (mg/dL, mmol/L)</th>
<th>Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL-cholesterol</td>
<td>&lt; 100 (&lt; 2.59)</td>
<td>Optimal</td>
</tr>
<tr>
<td></td>
<td>100-129 (2.59-3.34)</td>
<td>Near optimal/above optimal</td>
</tr>
<tr>
<td></td>
<td>130-159 (3.37-4.12)</td>
<td>High</td>
</tr>
<tr>
<td></td>
<td>160-189 (4.14-4.90)</td>
<td>Borderline high</td>
</tr>
<tr>
<td></td>
<td>&gt; 190 (&gt; 4.92)</td>
<td>Very high</td>
</tr>
<tr>
<td>HDL-cholesterol</td>
<td>&gt; 40 (&lt; 1.04)</td>
<td>Low</td>
</tr>
<tr>
<td></td>
<td>&gt; 60 (&gt; 1.55)</td>
<td>High</td>
</tr>
</tbody>
</table>

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cobas Lipid Panel

CHOL-TRIGL-HDL-LDL

The NCEP guidelines are based on serum values, and when classifying patients, serum or equivalent values should be used. Therefore, the NCEP recommends a factor of 1.03 to convert EDTA plasma values to serum values. Roche recommends that each laboratory validates its own conversion factor.

Non-high density lipoprotein

The NCEP ATP III gave the following recommendation: In persons with high triglycerides > 200 mg/dL (> 2.26 mmol/L), VLDL cholesterol should be combined with LDL-cholesterol, yielding non-HDL-cholesterol. The latter constitutes "atherogenic cholesterol" and should be a secondary target of therapy. After LDL goal is reached, secondary goal for non-HDL shall be set constituting "atherogenic cholesterol" and should be a secondary target of therapy. Instead, LDL-cholesterol is retained as the primary target of lipid-lowering therapy. The total cholesterol/HDL-cholesterol ratio is also not recommended as a secondary target of therapy. Treatment according to ratios will divert priority from specific lipoprotein fractions as targets of therapy.

Specific performance data

Representative performance data are given below. Results obtained in individual laboratories may differ. The bias at medical decision ranges:

Cholesterol:

\[ y = 1.000x - 0.110 \text{ mmol/L} \]

Mean bias (%) = -2.39 %

Triglycerides:

\[ y = 1.020x - 0.009 \text{ mmol/L} \]

Mean bias (%) = 0.45 %

High density lipoprotein:

\[ y = 1.056x - 0.080 \text{ mmol/L} \]

Mean bias (%) = 0.58 %

Bias at medical decision ranges:

low: 200 mg/dL (5.2 mmol/L): -2.1 %

high: 240 mg/dL (6.2 mmol/L): -1.8 %

Mean bias (%) = 0.45 %

Bias at medical decision ranges:

low: 150 mg/dL (1.7 mmol/L): 1.5 %

high: 200 mg/dL (2.26 mmol/L): 1.6 %

Method comparison

A comparison study with EDTA whole blood samples measured with cobas Lipid Panel (y) on the cobas b 101 instrument with respective methods on the cobas c 501 analyzer (x) gave the following correlations:

\[ y = 1.020x - 0.009 \text{ mmol/L} \]

Mean bias (%) = 0.45 %

Precision

Precision was determined using controls in a CLSI EP5-A2 protocol. Whole blood samples were measured using a modified CLSI protocol in 5 series of 4 replicates in one day. The following results were obtained:

Sample | Mean value mg/dL (mmol/L) | SD mg/dL (mmol/L) | CV % | SD mg/dL (mmol/L) | CV % |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Control/L1 (n = 84)</td>
<td>TC 145 (3.76)</td>
<td>2.0 (0.069)</td>
<td>1.4</td>
<td>1.3 (0.015)</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>TG 97 (1.10)</td>
<td>1.3 (0.015)</td>
<td>0.9</td>
<td>1.4 (0.037)</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td>HDL 42 (1.08)</td>
<td>1.3 (0.015)</td>
<td>1.0</td>
<td>1.4 (0.037)</td>
<td>1.8</td>
</tr>
<tr>
<td>Control/L2 (n = 84)</td>
<td>TC 269 (6.96)</td>
<td>4.8 (0.123)</td>
<td>1.8</td>
<td>1.3 (0.015)</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>TG 395 (4.46)</td>
<td>4.2 (0.048)</td>
<td>1.1</td>
<td>1.4 (0.049)</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>HDL 69 (1.78)</td>
<td>1.9 (0.048)</td>
<td>1.1</td>
<td>2.1 (0.055)</td>
<td>1.1</td>
</tr>
<tr>
<td>Whole blood (n = 20)</td>
<td>TC 166 (4.29)</td>
<td>3.1 (0.080)</td>
<td>1.9</td>
<td>1.2 (0.045)</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>TG 336 (3.80)</td>
<td>4.0 (0.045)</td>
<td>1.2</td>
<td>1.4 (0.035)</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>HDL 38 (0.97)</td>
<td>0.9 (0.023)</td>
<td>1.9</td>
<td>2.4 (0.050)</td>
<td>1.3</td>
</tr>
</tbody>
</table>

References


17 Bersot TP, Pépin GM, Mahley RW. Risk determination of dyslipidemia in populations characterized by low levels of high-density lipoprotein cholesterol. Am Heart J 2003;146:1052-9.


A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Symbols
Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard.

SYSTEM

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Significant additions or changes are indicated by a change bar in the margin.

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