Related topics
Cholesterol, lipoproteins, photometry, hyperlipidaemia

Principle
Cholesterol is a constituent of all biological membranes and thus an essential lipid. Increased cholesterol levels represent a major risk factor for arteriosclerotic angiopathy, particularly coronary heart disease. The cholesterol concentration in a blood sample is determined photometrically by colour indicator reaction.

Equipment

<table>
<thead>
<tr>
<th>Student Basic Set</th>
<th>1 Disposable stirring spatulas, 500 pcs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Microliter pipette 10 – 100 µl</td>
<td>47141-03</td>
</tr>
<tr>
<td>1 Microliter pipette 100 – 1000 µl</td>
<td>47141-05</td>
</tr>
<tr>
<td>1 Test tube rack, PP, 12 places</td>
<td>46235-00</td>
</tr>
<tr>
<td>1 Graduated pipette 10 ml, scale division</td>
<td>36600-00</td>
</tr>
<tr>
<td>1 Pipette filler</td>
<td>47127-01</td>
</tr>
<tr>
<td>1 Digital stop watch</td>
<td>24025-00</td>
</tr>
<tr>
<td>1 Laboratory beaker, 100 ml, PP</td>
<td>36011-01</td>
</tr>
<tr>
<td>1 Laboratory pencil, waterproof</td>
<td>38711-00</td>
</tr>
<tr>
<td>1 Pipette tips 2 - 200 µl in holders, 96 pcs.</td>
<td>47148-11</td>
</tr>
<tr>
<td>1 Pipette tips 50 - 1000 µl in holders, 100 pcs.</td>
<td>47148-12</td>
</tr>
<tr>
<td>1 Disposable gloves, M, latex, 100 pcs.</td>
<td>46395-00</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Lab Equipment</th>
<th>1</th>
<th>1</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Photometer</td>
<td>35602-99</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Semi-micro cuvettes</td>
<td>35662-10</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Cuvette rack</td>
<td>35661-10</td>
<td></td>
<td></td>
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<tr>
<td>Cuvette rack</td>
<td>35661-10</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Chemicals</th>
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</thead>
<tbody>
<tr>
<td>Water, distilled, 5 l</td>
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</tbody>
</table>

Additionally, the following reagents are required:
- Cholesterol reagent
- Control sample TruLab N and P
Note: The reagents and control samples used for this experiment are listed in the Appendix. Please note! Always observe the preliminary remarks when performing this experiment.

Tasks
1. Determine the cholesterol content of a serum sample and a control sample.
2. Calculate the associated deviation of the individual measured value of the control sample.

Procedure

Preparation of reagent
The reagent is ready for use
The standard is ready for use. It is included in the package with the reagent.
Bring reagent to room temperature, following the instructions in the package leaflet.

Material for the analysis
Serum - Please refer to Chapter “Pre-Analytics”. TruLab P or N are used as control samples. They are lyophilised. Dissolve in distilled water following instructions in the package leaflet (refer to chapter “Preliminary Remarks”).

Measuring workflow
The temperature should be 20 – 25°C.

Pipetting scheme a total of 4 samples are measured as follows:
- Blank value
- Standard sample
- Control sample
- Patient sample

10 µl of the respective sample or, in case of the reagent blank value, distilled water are mixed with 1000 µl of the reagent directly in the graduated cuvette.

After incubating at room temperature for 10 minutes, the extinction is to be measured against the reagent blank value within 60 minutes.

First, the reagent blank value has to be set.
- Switch on device: on/off
- Set wave length: 550 nm
- Place cuvette for blank value into the measuring cell and start reference measurement: Press button “R”

Measurement of the samples:
- Place sample in measuring cell, start measurement: Press button “T”
- Read extinction
- Switch off device: on/off
**Basics**

Cholesterol, as a constituent of all biological membranes, is an essential lipid. Apart from that, it is the biosynthesis precursor of numerous steroid hormones, of vitamin D and bile acids. Due to their low solubility in water, cholesterols and cholesterol esters will only be found in the plasma as forms attached to various lipoproteins.

Cholesterol is taken up with the food, but also synthesized endogenously. It is excreted via the skin, the gall and the intestines; reabsorption via the biliary cycle is possible as well. Cholesterol determination serves the purpose of early diagnosis of the risk for arteriosclerosis, of monitoring therapies with lipid-lowering drugs as well as the diagnosis of hyperlipoproteinemic and hypolipoproteinemic diseases.

When high triglyceride and/or cholesterol values are measured in the serum of a patient after 12 hours of alimentary abstinence, hyperlipoproteinaemia is proven. Hyperlipoproteinaemias are metabolic diseases based on increased synthesis or delayed decomposition of lipoproteins transporting cholesterol and triglycerides in the blood plasma. Increased plasmalipoprotein concentrations cause arteriosclerosis. In case of hereditary causes, these diseases are called primary hyperlipidaemias. When the increase of blood lipids occurs within the framework of a disease (e.g. diabetes mellitus, kidney disease, alcoholism), it is called secondary hyperlipidaemia. Secondary hyperlipidaemias occur more frequently.

Hyperhomocysteinaemia is rated as an independent risk factor for atherosclerotic angiopathies. Increased homocystein levels lead to damages at the endothelium of blood vessels. It occurs mostly in case of vitamin B12, -B2 and B6 deficiencies as well as folic acid deficiency.

Further diagnostics: Triglycerides, lipoprotein electrophoresis, homocysteine

**Test principle**

Following enzymatic hydrolysis of the cholesterol esters, the entire cholesterol complex is oxidized by oxygen from the air. In this process, hydrogen peroxide is generated, which, in combination with phenol and 4-aminophenazon under the effect of peroxidase, produces a red dye, the extinction of which is determined at 546 nm. The intensity of its colour is directly proportional to the cholesterol concentration and is calculated with the aid of a standard.

**Evaluation**

**Task 1**

The cholesterol concentration can be determined directly from the extinction of the samples, via the standard. As the standard is quoted in mg/dl, conversion of the concentration into SI-units (mmol/l) is to be carried out subsequently.

\[
c_{\text{Cholesterol}}[\text{mg/dl}] = \frac{\Delta E_{\text{Sample}}}{\Delta E_{\text{Standard}}} \cdot c_{\text{Standard}}[\text{mg/dl}]
\]

\[
c_{\text{Cholesterol}}[\text{mg/dl}] \cdot 0.02586 = c_{\text{Cholesterol}}[\text{mmol/l}]
\]

**Example:**

\[
c_{\text{Standard}} = 200 \text{ mg/dl}, \Delta E_{\text{Sample}} = 0.17, \Delta E_{\text{Standard}} = 0.25
\]

\[
c_{\text{Control}}[\text{mg/dl}] = \frac{0.17}{0.25} \cdot 200 \text{ mg/dl} = 136 \text{ [mg/dl]}
\]
Task 2: Calculate the deviation of the individual measured value of the control sample (KoPEM).

The deviation of the individual measured value of the control sample is used to verify whether errors occurred in the experiment (according to Guidelines of the Federal Medical Council on quality assurance in medical laboratory tests. Please observe statutory provisions in your country - see preliminary remarks). For this purpose, a control sample is measured in addition to the patient sample.

The value of the control sample determined in the experiment is the so-called Actual Value. But we also know the true value of the control sample, called Target value. It is indicated in the package leaflet of the control serum (Trulab P or N). The following formula is used to calculate the deviation of the individual measured value of the control sample (abbr. KoPEM) from the determined actual value and the known target value in %:

\[
KoPEM[\%] = \frac{\text{Target} - \text{Actual}}{\text{Target}} \cdot 100
\]

Exemplary calculation:

Concentration of control sample Actual: 136 mg/dl
Concentration of control sample Target: 146 mg/dl

\[
KoPEM[\%] = \frac{146 - 136}{146} \cdot 100 = 6.8\%
\]

Following this, the deviation of the individual measured value of the control sample from the target value is compared to the limits permitted under general rules in the respective country. In the Federal Republic of Germany, this limit for cholesterol is indicated as +/ -7.0 % in Table B 1a RiliBÄK. [Guidelines of the German Federal Medical Council on quality assurance in medical laboratory tests] In our example, the individual measured value of the control sample fulfils this default. The patient may thus be assessed.

Please note: Please observe statutory provisions in your respective country!
Please refer to chapter “Preliminary Remarks”.

Reference values
Adults <240 mg/dl

Note
Error sources: Highly lipemic, icteric or haemolytic samples.

Questions
- Why does cholesterol require a transport protein in the body?
  Lipids are not soluble in water, for this reason they need a transportation molecule in the blood stream.

- What has to be kept in mind when withdrawing blood?
  Standardized blood withdrawal: 12 hours of alimentary abstinence

- For which purposes does the body need cholesterol?
  Building membranes, synthesis of steroid hormones, production of bile acids
Lab Report

Task 1
Please enter your measured results in the following table

<table>
<thead>
<tr>
<th></th>
<th>Extinction</th>
<th>Concentration [mg/dl]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank value</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard (according</td>
<td></td>
<td></td>
</tr>
<tr>
<td>to package)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control sample TruLab N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient 1</td>
<td></td>
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</tr>
</tbody>
</table>

Task 2
Record the deviation of the KoPEM and discuss the result.
_____________________________________________________________________________
_____________________________________________________________________________

Questions

- Why does cholesterol require a transport protein in the body?
_____________________________________________________________________________
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_____________________________________________________________________________
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- What has to be kept in mind when withdrawing blood?
_____________________________________________________________________________
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_____________________________________________________________________________
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- For which purposes does the body need cholesterol?