**TEST PRINCIPLE**

Determination of cholesterol after enzymatic hydrolysis and oxidation [3,4]. The colorimetric indicator is quinonemine which is generated from 4-aminoantipyrine and phenol by hydrogen peroxide under the catalytic action of peroxidise (Trinder’s reaction) [3].

Cholesterol ester + H₂O₂ → Cholesterol + fatty acid

Cholesterol + O₂ + 4 Aminoantipyrine → 2 H₂O₂ + Phenol + 4 Aminoantipyrine → Quinonemine + 4 H₂O₂

The intensity of the pink/red colour is proportional to the Cholesterol concentration in the sample.

**REAGENT COMPOSITION**

**COMPONENTS**

- NaCl solution (9 g/L)
- Good’s buffer, pH 6.7
- Phenol
- 4-Aminoantipyrine
- Cholesterol esterase (CHE)
- Cholesterol oxidase (CHO)
- Peroxidase (POD)

**CONCENTRATIONS**

- Good’s buffer, pH 6.7: 50 mmol/L
- Phenol: 5 mmol/L
- 4-Aminoantipyrine: 0.3 mmol/L
- Cholesterol esterase (CHE): ≥ 200 U/L
- Cholesterol oxidase (CHO): ≥ 50 U/L
- Peroxidase (POD): ≥ 3 kU/L

**REAGENT PREPARATION**

The reagent is ready to use.

**REAGENT STABILITY AND STORAGE**

Conditions:  protect from light
- close immediately after use
- avoid contamination
- do not freeze the reagent
- Storage: at 2 – 8 °C
- Stability: up to the indicated expiration date

**Note:** The measurement is not influenced by occasionally occurring colour changes, as long as the absorbance of the reagent is < 0.3 at 546 nm.

**SAMPLE STABILITY AND STORAGE [6]**

Stability:  at 20 – 25 °C 7 days
- at 4 – 8 °C 7 days
- at -20 °C 3 months

**MATERIALS REQUIRED BUT NOT PROVIDED**

- 
  - NaCl solution (9 g/L)
  - General laboratory equipment

**STANDARD**

- (not included in the kit – has to be ordered separately)
  - Concentration: 200 mg/dL (5.20 mmol/L)
  - Storage: 2 – 25 °C
  - Stability: up to the indicated expiration date

Close immediately after use! Avoid contamination! Protect from light.

**MANUAL TEST PROCEDURE**

Bring reagents and samples to room temperature.

<table>
<thead>
<tr>
<th>Pipette into test tubes</th>
<th>Blank</th>
<th>Std./Cal.</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent</td>
<td>1000 µL</td>
<td>1000 µL</td>
<td>1000 µL</td>
</tr>
<tr>
<td>Sample</td>
<td>-</td>
<td>-</td>
<td>10 µL</td>
</tr>
<tr>
<td>Standard/Calibrator</td>
<td>-</td>
<td>10 µL</td>
<td>-</td>
</tr>
<tr>
<td>Dist water</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Mix. Incubate 10 minutes at 37 °C or 20 minutes at 20 – 25 °C. Read absorbance of sample and Std./Cal. within 60 minutes against the reagent blank.

**CALCULATION**

Cholesterol [mg/dL] = A Sample x conc. Std./Cal [mg/dL] x Std/Cal
UNIT CONVERSION

mg/dL x 0.02586 = mmol/L

REFERENCE RANGE [5] *

<table>
<thead>
<tr>
<th></th>
<th>≤ 200 mg/dL (5.2 mmol/L)</th>
<th>200 – 240 mg/dL (5.2 – 6.2 mmol/L)</th>
<th>&gt; 240 mg/dL (&gt; 6.2 mmol/L)</th>
</tr>
</thead>
</table>

* Each laboratory should check if the reference ranges are transferable to its own patient population and determine own reference ranges if necessary.

Clinical Interpretation
The European Task Force on Coronary Prevention recommends to lower Total Cholesterol concentration to less than 190 mg/dL (5.0 mmol/L) and LDL-cholesterol to less than 115 mg/dL (3.0 mmol/L) [2].

PERFORMANCE CHARACTERISTICS

LINEARITY, MEASURING RANGE
The test has been developed to determine cholesterol concentrations within a measuring range from 3 – 750 mg/dL (0.08 – 19.4 mmol/L). If values exceed this range, samples should be diluted 1+ 4 with NaCl solution (9 g/L) and the result multiplied by 5.

SENSITIVITY/LIMIT OF DETECTION
The lower limit of detection is 3 mg/dL (0.08 mmol/L).

PRECISION (at 37°C)

<table>
<thead>
<tr>
<th></th>
<th>Mean [mg/dL]</th>
<th>SD [mg/dL]</th>
<th>CV [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intra-assay n = 20</td>
<td>Sample 1: 108</td>
<td>1.76</td>
<td>1.62</td>
</tr>
<tr>
<td></td>
<td>Sample 2: 236</td>
<td>1.45</td>
<td>0.61</td>
</tr>
<tr>
<td></td>
<td>Sample 3: 254</td>
<td>1.57</td>
<td>0.62</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Mean [mg/dL]</th>
<th>SD [mg/dL]</th>
<th>CV [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inter-assay n = 20</td>
<td>Sample 1: 104</td>
<td>1.19</td>
<td>1.14</td>
</tr>
<tr>
<td></td>
<td>Sample 2: 211</td>
<td>2.57</td>
<td>1.22</td>
</tr>
<tr>
<td></td>
<td>Sample 3: 245</td>
<td>2.28</td>
<td>0.93</td>
</tr>
</tbody>
</table>

SPECIFICITY/INTERFERENCES
no interference up to:
- Ascorbic acid: 5 mg/dL
- Bilirubin: 20 mg/dL
- Hemoglobin: 200 mg/dL
- Triglycerides: 2000 mg/dL

For further information on interfering substances refer to Young DS [7].

METHOD COMPARISON
A comparison between Dialab Cholesterol (y) and a commercially available test (x) using 78 samples gave following results: y = 1.00 x – 2.50 mg/dL; r= 0.995.

CALIBRATION
The assay requires the use of a Cholesterol standard or calibrator.
We recommend the Dialab Cholesterol Standard or the multi calibration serum Diacal Auto. The assigned values of the calibrator have been made traceable to the reference method gas chromatography – isozone dilution mass spectrometry (CG-IDMS).

QUALITY CONTROL
All control sera with Cholesterol values determined by this method can be used.
We recommend the Dialab lipid control sera Diacon Lipids and Diacon Lipids High and the Dialab multi control sera Diacon N (with values in the normal range) and Diacon P (with values in the pathological range). Each laboratory should establish corrective action in case of deviations in control recovery.

AUTOMATION
Special applications for automated analysers can be made on request.

WARNINGS AND PRECAUTIONS
1. The reagent contains sodium azide (0.95 g/L) as preservative. Do not swallow! Avoid contact with skin and mucous membranes.
   H317: May cause an allergic skin reaction.
   H319: Causes serious eye irritation.
   P280: Wear protective gloves/protective clothing/eye protection/face protection.
   P302+P352: If on skin: Wash with plenty of soap and water.
   P337+P313: If eye irritation persists: Get medical advice/attention.
3. In very rare cases, samples of patients with gammopathy might give falsified results [8].
4. N-acetylcysteine (NAC), acetaminophen and metamizole medication leads to falsely low results in patient samples.
5. Please refer to the safety data sheet and take the necessary precautions for the use of laboratory reagents.
6. For diagnostic purposes, the results should always be assessed with the patient’s medical history, clinical examinations and other findings.
7. For professional use only!

WASTE MANAGEMENT
Please refer to local legal requirements.

REFERENCES