Quantitative determination of cholesterol

IVD

Store at 2-8°C

PRINCIPLE OF THE METHOD

The cholesterol present in the sample originates a coloured complex, according to the following reaction:

\[
\text{Cholesterol esters + H}_2\text{O} \rightarrow \text{CHE} \rightarrow \text{Cholesterol + fatty acids}
\]

\[
\text{Cholesterol + O}_2 \rightarrow \text{CHOD} \rightarrow 4\text{-Cholestenone + H}_2\text{O}
\]

2 H\text{O} + Phenol + 4-Aminophenazine \rightarrow \text{POD} \rightarrow \text{Quinonimine + 4H}_2\text{O}

The intensity of the color formed is proportional to the cholesterol concentration in the sample.\(^2\)

CLINICAL SIGNIFICANCE

Cholesterol is a fat-like substance that is found in all body cells. The liver makes all of the cholesterol the body needs to form cell membranes and to make certain hormones.

The determination of serum cholesterol is one of the important tools in the diagnosis and classification of lipemia. High blood cholesterol is one of the major risk factors for heart disease.\(^3,4\)

Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

REAGENTS

| R 1 Buffer | PIPES pH 6.9 | 90 mmol/L |
| R 2 Enzymes | Cholesterol esterase (CHE) | 300 U/L |
| | Cholesterol oxidase (CHOD) | 300 U/L |
| | Peroxidase (POD) | 1250 U/L |
| | 4 – Aminophenazine (4-AP) | 0.4 mmol/L |

CHOLESTEROL CAL

Cholesterol aqueous primary standard 200 mg/dL

PREPARATION

Working reagent (WR): Dissolve (\(\rightarrow\)) the contents of one vial R 2 Enzymes in one bottle of R 1 Buffer.

Cap and mix gently to dissolve contents.

(WR) is stable: 4 moths at 2-8°C or 40 days at 15-25°C.

Avoid direct sunlight.

STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8°C, protected from light and contaminations prevented during their use.

Do not use reagents after the expiration date.

Signs of reagent deterioration:

- Presence of particles and turbidity.
- Blank absorbance (A) at 505 nm \(\geq 0.1\).

ADDITIONAL EQUIPMENT

- Spectrophotometer or colorimeter measuring at 505 nm (500-550).
- Matched cuvettes 1.0 cm light path.
- General laboratory equipment.

SAMPLES

Serum or plasma:\(^2,5\): Stability of the sample for 7 days at 2-8°C or freezing at –20°C will keep samples stable for a 3 months.

PROCEDURE

1. Assay conditions:
   - Wavelength: \(505 \text{ nm (500-550)}\)
   - Cuvette: \(1 \text{ cm light path}\)
   - Temperature: \(37°C/15-25°C\)

2. Adjust the instrument to zero with distilled water.

3. Pipette into a cuvette:

<table>
<thead>
<tr>
<th>WR (mL)</th>
<th>Blank</th>
<th>Standard</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td></td>
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</table>

<table>
<thead>
<tr>
<th>Standard (mmol/L)</th>
<th>10</th>
</tr>
</thead>
</table>

   | Sample (mL) | 10 |

4. Mix and incubate for 5 min. at 37°C or 10 min. at room temperature.

5. Read the absorbance (A) of the samples and Standard, against the Blank. The colour is stable for at least 60 minutes.

CALCULATIONS

\[
(A)\text{Sample} - (A)\text{Blank} = 200 \times (\text{Standard conc}) = \text{mg/dL cholesterol in the sample}
\]

Conversion factor: mg/dL \(\times 0.0258 = \text{mmol/L}\).

QUALITY CONTROL

Control sera are recommended to monitor the performance of assay procedures: SPINTROL H Normal and Pathologic (Ref. 1002120 and 1002210).

If control values are found outside the defined range, check the instrument, reagents and calibrator for problems.

Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES

Risk evaluation\(^5,6\): Normal \(200-239 \text{ mg/dL}\), Borderline \(\geq 240 \text{ mg/dL}\), High

These values are for orientation purpose; each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS

Measuring range: From detection limit of 0 mg/dL to linearity limit of 900 mg/dL.

If the results obtained were greater than linearity limit, dilute the sample 1/2 with NaCl 9 g/L and multiply the result by 2.

Precision:

<table>
<thead>
<tr>
<th>Intra-assay (n=20)</th>
<th>Inter-assay (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (mg/dL)</td>
<td>90.4</td>
</tr>
<tr>
<td>SD</td>
<td>1.15</td>
</tr>
<tr>
<td>CV (%)</td>
<td>1.27</td>
</tr>
</tbody>
</table>

Sensitivity: 1 mg/dL = 0.00152 A.

Accuracy: Results obtained using SPINREACT reagents (y) did not show systematic differences when compared with other commercial reagents (x).

The results obtained using 50 samples were the following: Correlation coefficient \((r) = 0.99541\).

Regression equation: \(y = 0.95293x – 3.020\).

The results of the performance characteristics depend on the analyzer used.

INTERFERENCES

Hemoglobin up to 5 g/L and bilirubin up to 10 mg/dL, do not interfere.\(^2,6\)

A list of drugs and other interfering substances with cholesterol determination has been reported.\(^3,4\)

NOTES

1. CHOLESTEROL CAL: Proceed carefully with this product because due its nature it can get contaminated easily.
2. LCF (Lipid Clearing Factor) is integrated in the reagent.
3. Calibration with the aqueous Standard may cause a systematic error in procedures: these cases, it is recommended to use a serum Calibrator.
4. Use clean disposable pipette tips for its dispensation.
5. SPINREACT has instruction sheets for several automatic analyzers.

BIBLIOGRAPHY