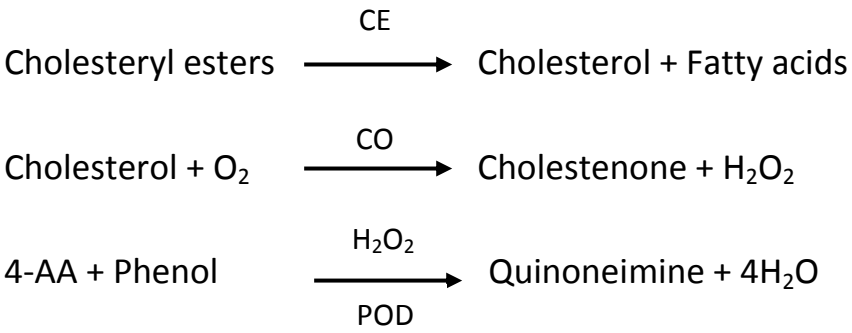
Cholesterol Determination

1. Clinical importance.

Cholesterol exists in the human blood as a free sterol and in an esterified form. The knowledge of the plasma level of lipids (cholesterol and triglycerides) together with lipoproteins of high and low density (HDL and LDL) aids in the detection of many conditions bound to metabolic disorders of high risk. The imbalance in the level of lipoproteins in plasma leads to hyperlipoproteinemias, a group of disorders that affects lipid levels in serum, causing coronary heart disease (CHD) and atherosclerosis, conditions in which the cholesterol levels are important tools in their diagnosis and classification. Jaundice of the obstructive type usually is accompanied by an elevated total serum cholesterol with a normal ester fraction. Diabetes, hypothyroidism, and certain types of kidney disease are other disorders that may exhibit the same cholesterol disturbance. Low total cholesterol values with normal ester fractions are noted mainly in hyperthyroidism and malnutrition.

2. Principle.

This method for the measurement of total cholesterol in serum involves the use of three enzymes: cholesterol esterase (CE), cholesterol oxidase (CO) and peroxidase (POD). In the presence of the former the mixture of phenol and 4-aminoantipyrine (4-AA) are condensed by hydrogen peroxide to form a quinoneimine dye proportional to the concentration of cholesterol in the sample.



3. Reagents preparation.

All of the reagents are prepared by linear S.L.U. Company as a kit, as follows:

Reagent 1: (Monoreagent). PIPES 200 mmol/L pH 7.0, sodium cholate 1 mmol/L, cholesterol esterase > 250 U/L, cholesterol oxidase > 250 U/L, peroxidase > 1 KU/L, 4-aminoantipyrine 0.33 mmol/L, phenol 4 mmol/L, non-ionic tensioactives 2 g/L (w/v).
Reagent 2: (Cholesterol standard). Cholesterol 200 mg/dL (5.18 mmol/L).

All the kit compounds are stable until the expiry date stated on the label. Do not use reagents over the expiration date. Store the vials tightly closed, protected from light and prevented contaminations during the use. Store the kit at 2-8°c. Avoid contamination and recap the vials immediately after use.
Discard If appear signs of deterioration:

- Presence of particles and turbidity.

- Blank absorbance (A) at 500 nm > 0.200 in 1cm cuvette.

The monoreagent and the standard are ready to use.

4. Samples.

Serum, EDTA or heparinized plasma free of hemolysis. Cholesterol in serum or plasma is stable up to 5 days at 2-8°c. and for a few months at –20°c.

5. Procedure.

1. Bring reagents and samples to room temperature.
2. In disposable test tube add 1 ml of monoreagent (reagent 1) and label the tube as sample.
3. Add 10 µl of serum to the sample tube.
4. In other disposable test tube add 1 ml of monoreagent (reagent 1) and label the tube as standard.
5. Add 10 µl of standard solution (reagent 2).
6. Mix and let the tubes stand for 10 minutes at room temperature or 5 minutes at 37°c.
7. Read the absorbance (A) of the samples and the standard at 500 nm against the reagent blank. The color is stable for 30 minutes protected from light.
8. Calculate the concentration of triglyceride from the equation:

$$\text{cholesterol concentration mg/dl} = \frac{A_{\text{sample}}}{A_{\text{standard}}} \times C_{\text{standard}}$$

A: Absorbance, C: concentration.

Note: Samples with concentrations higher than 600 mg/dl should be diluted 1:2 with saline and assayed again. Multiply the results by 2.

The clinical values for triglyceride concentration are:

Desirable: < 200 mg/dL.
Borderline/high: 200-239 mg/dL.
High: >240 mg/dL.