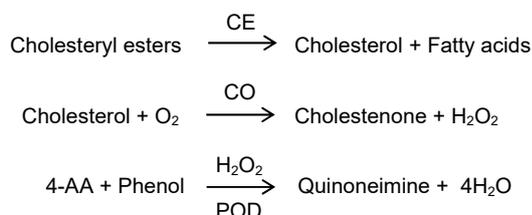


CHOLESTEROL MR

REF 1118005 2 x 50 mL CONTENTS R1. Reagent 2 x 50 mL CAL. Standard 1 x 3 mL	REF 1118010 4 x 100 mL CONTENTS R1. Reagent 4 x 100 mL CAL. Standard 1 x 3 mL	REF 1118015 4 x 250 mL CONTENTS R1. Reagent 4 x 250 mL CAL. Standard 1 x 3 mL	<h2>CHOLESTEROL MR</h2> TOTAL <i>Enzymatic colorimetric method</i> ENDPOINT
For <i>in vitro</i> diagnostic use only			

PRINCIPLE

This method for the measurement of total cholesterol^{1,2} 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.



REAGENT COMPOSITION

R1 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). Biocides.

CAL Cholesterol standard. Cholesterol 200 mg/dL (5.18 mmol/L). Organic matrix based primary standard. Concentration value is traceable to Standard Reference Material 909b.

STORAGE AND STABILITY

Store at 2-8°C.
 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.

Discard if appear signs of deterioration:

- Presence of particles and turbidity.
- Blank absorbance (A) at 500 nm > 0.200 in 1cm cuvette.

REAGENT PREPARATION

The Monoreagent and the Standard are ready-to-use.

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.

INTERFERENCES

- Lipemia (intralipid 5 g/L) may affect the results.
- Bilirubin (40 mg/dL) does not interfere.
- Hemoglobin (> 1 g/L) may affect the results.
- Other drugs and substances may interfere³.

MATERIALS REQUIRED

- Photometer or colorimeter capable of measuring absorbance at 500 ± 10 nm.
- Constant temperature incubator set at 37°C.
- Pipettes to measure reagent and samples.

PROCEDURE

1. Bring reagents and samples to room temperature.
2. Pipette into labelled tubes:

TUBES	Blank	Sample	CAL. Standard
R1. Monoreagent	1.0 mL	1.0 mL	1.0 mL
Sample	-	10 µL	-
CAL. Standard	-	-	10 µL

3. Mix and incubate the tubes 10 minutes at room temperature or 5 minutes at 37°C.
4. Read the absorbance (A) of the samples and the standard at 500 nm against the reagent blank.

The color is stable for at least 30 minutes protected from light.

CALCULATIONS

$$\frac{A_{\text{Sample}}}{A_{\text{Standard}}} \times C_{\text{Standard}} = \text{mg/dL total cholesterol}$$

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

If results are to be expressed as SI units apply:
 mg/dL x 0.0259 = mmol/L



REFERENCE VALUES⁴

Updated clinical values of total cholesterol used to classify risk groups.

Total Cholesterol	Risk Classification
< 200 mg/dL (< 5.18 mmol/L)	Desirable
200-239 mg/dL (5.18-6.2 mmol/L)	Borderline high
> 240 mg/dL (> 6.2 mmol/L)	High

QUALITY CONTROL

The use of a standard to calculate results allows to obtain an accuracy independent of the system or instrument used.

To ensure adequate quality control (QC), each run should include a set of controls (normal and abnormal) with assayed values handled as unknowns.

REF 1980005 HUMAN MULTISERA NORMAL
Borderline level of cholesterol. Assayed.

REF 1985005 HUMAN MULTISERA ABNORMAL
Elevated level of cholesterol. Assayed.

If the values are found outside of the defined range, check the instrument, reagents and procedure.

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

CLINICAL SIGNIFICANCE

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.

ANALYTICAL PERFORMANCE

- **Detection Limit** : 1.20 mg/dL

- **Linearity** : Up to 600 mg/dL

- **Precision**:

mg/dL	Within-run		Between-run	
Mean	179.3	302.2	179.3	302.2
SD	1.40	1.35	5.10	9.12
CV%	0.77	0.45	2.84	3.02
N	10	10	10	10

- **Sensitivity** : 2 mA / mg/dL cholesterol.

- **Correlation**: This assay (y) was compared with a similar commercial method (x). The results were:

$$N = 60 \quad r = 0.97 \quad y = 1.00x + 0.77$$

The analytical performances have been generated using on automatic instrument. Results may vary depending on the instrument.

NOTES

- This method may be used with different instruments. Any application to an instrument should be validated to demonstrate that results meet the performance characteristics of the method. It is recommended to validate periodically the instrument. Contact to the distributor for any question on the application method.
- Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.

REFERENCES

- Allain, C.C., Poon, L.S., Clau, C.S.G, Richmond, W and Fu, P.D. Clin. Chem. 20 : 470 (1974).
- Richmond, W. Ann. Clin. Biochem. 29 : 577 (1992).
- Young DS. Effects of drugs on clinical laboratory tests, 5th ed. AACC Press, 2000.
- SPECIAL REPORT. Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). JAMA. 285 : 2486 (2001).

Further hints

- SPECIAL REPORT (ATP III) available at:
[http:// www.nhlbi.nih.gov](http://www.nhlbi.nih.gov).
- An autoevaluation about the risk of heart disease is available at :
[time.com/ cholesterol](http://time.com/cholesterol)

