CHOLESTEROL

COD 12505 10 x 50 mL
Only for in vitro use in the clinical laboratory

Automated Systems

BioSystems

CHOLESTEROL
CHOLESTEROL OXIDASE/PEROXIDASE

INTENDED USE

Reagent for the measurement of cholesterol concentration in human serum or plasma. The obtained values are useful as an aid in the risk of suffering clinical manifestations of atherosclerosis.

This reagent is for use in the BioSystems A25 and A15 analyzers or in other analyzer with similar performance characteristics.

CLINICAL SIGNIFICANCE

Cholesterol is a steroid of high molecular weight and possesses the cyclopentanophenanthrene skeleton. Dietary cholesterol is partially absorbed and it is also synthesized by the liver and other tissues. Cholesterol is transported in plasma by lipoproteins. It is excreted unchanged into bile or after transformation to bile acids.

Increased total cholesterol values are associated with a progressively escalating risk of atherosclerosis and coronary artery disease[1,2].

Clinical diagnosis should not be made on the findings of a single test result, but should integrate both clinical and laboratory data.

PRINCIPLE OF THE METHOD

Free and esterified cholesterol in the sample originates, by means of the coupled reactions described below, a coloured complex that can be measured by spectrophotometry[3,4].

$$\text{Cholesterol ester} + H_2O \xrightarrow{\text{chol. esterase}} \text{Cholesterol} + \text{Fatty acid}$$

$$\text{Cholesterol} + \tfrac{1}{2}\, O_2 + H_2O \xrightarrow{\text{chol. oxidase}} \text{Cholestenone} + H_2O_2$$

$$2\, H_2O_2 + 4 - \text{Aminoantipyrine} + \text{Phenol} \xrightarrow{\text{peroxidase}} \text{Quinoneimine} + 4\, H_2O$$

CONTENTS AND COMPOSITION

A. Reagent. 10 x 50 mL. Pipes 35 mmol/L, sodium cholate 0.5 mmol/L, phenol 28 mmol/L, cholesterol esterase > 0.2 U/mL, cholesterol oxidase > 0.1 U/mL, peroxidase > 0.8 U/mL, 4-aminoantipyrine 0.5 mmol/L, pH 7.0.

STORAGE AND STABILITY

Store at 2-8ºC.

Components are stable once opened until the expiry date marked in the label if they are stored well closed and care is taken to prevent contamination during their use.

On board stability: Reagents open and kept in the refrigerated compartment of the analyzer are stable 2 months.

Indications of deterioration: Absorbance of the blank over the limit indicated in "Test Parameters".

ADDITIONAL MATERIALS REQUIRED (NOT PROVIDED)

Biochemistry Calibrator (BioSystems cod. 18011) or Biochemistry Calibrator Human (BioSystems cod. 18044).

REAGENT PREPARATION

Reagent is provided ready to use.

SAMPLES

Serum or plasma collected by standard procedures.

Cholesterol is stable for 7 days at 2-8ºC. Heparin, EDTA, oxalate and fluoride may be used as anticoagulants.

CALIBRATION

A reagent blank should be done every day and a calibration at least every 2 months, after reagent lot change or as required by quality control procedures.

QUALITY CONTROL

It is recommended to use the Biochemistry Control Serum level I (cod. 18005, 18009 and 18042) and II (cod. 18007. 18010 and 18043) to verify the accuracy of the measurement procedure.

Each laboratory should establish its own internal Quality Control scheme and procedures for corrective action if control results are not within the acceptable limits.

REFERENCE VALUES

The following uniform cut-off points have been established by the US National Cholesterol Education Program and have also been adopted in many other countries for the evaluation of coronary artery disease risk[5].

Up to 200 mg/dL = 5.2 mmol/L	Desirable
200-239 mg/dL = 5.2-6.21 mmol/L	Borderline High
> 240 mg/dL = > 6.24 mmol/L	High

METROLOGICAL CHARACTERISTICS

The metrological characteristics described below have been obtained using an A25 analyzer. Results are similar with A15.

- Detection limit: 0.9 mg/dL = 0.023 mmol/L.
- Linearity limit: 1000 mg/dL = 26 mmol/L.
- Precision:

Mean concentration	Repeatability (CV)	Within-laboratory (CV)
142 mg/dL = 3.68 mmol/L	1.9 %	3.1 %
242 mg/dL = 6.27 mmol/L	1.5 %	3.5 %

- Trueness: Results obtained with this reagent did not show systematic differences when compared with reference reagents. Details of the comparison experiments are available on request.

LIMITATIONS OF THE PROCEDURE

- Interferences: Hemolysis (hemoglobin up to 500 mg/dL), bilirubin (up to 10 mg/dL) and lipemia (triglycerides up to 1000 mg/dL) do not interfere. Ascorbic acid (up to 6.25 mg/dL) does not interfere. Other drugs and substances may interfere[6].

BIBLIOGRAPHY

1. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, 4th ed. Burtis CA, Ashwood ER, Bruns DE. WB Saunders Co, 2005.
2. Friedman and Young. Effects of disease on clinical laboratory tests, 4th ed. AACC Press, 2001.
3. Allain CC, Poon LS, Chan CSG, Richmond W and Fu PC. Enzymatic determination of total serum cholesterol. *Clin Chem* 1974; 20: 470-475.
4. Meiattini F, Prencipe L, Bardelli F, Giannini G and Tarli P. The 4-hydroxybenzoate/4-aminophenazone chromogenic system used in the enzymic determination of serum cholesterol. *Clin Chem* 1978; 24: 2161-2165.
5. National Cholesterol Educarion Program Expert Panel. Third report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (ATP III). NIH Publication. Bethesda: National Heart, Lung, and Blood Institute; 2001.
6. Young DS. Effects of drugs on clinical laboratory tests, 5th ed. AACC Press, 2000.

TEST PARAMETERS

These reagents may be used in several automatic analyzers. Specific instructions for application in many of them are available on request.

R1: use Reagent A.

	A25	A15
GENERAL		
Name	**CHOLESTEROL**	**CHOLESTEROL**
Sample type	SER	SER
Analysis mode	endpoint mon.	endpoint mon.
Units	mg/dL	mg/dL
Turbidimetry test	no	no
Decimals	0	0
Type of reaction	increasing	increasing
PROCEDURE		
Reading mode	bichrom.	bichrom.
Main filter	505	505
Reference filter	670	670
Sample	3	3
Vol. R1	300	300
Vol. R2	-	-
Washing	1.2	1.2
Reading 1 (cycle)	21	14
Reading 2 (cycle)	-	-
Reagent 2 (cycle)	-	-
Predilution factor	-	-
CALIBRATION AND BLANK		
Calibration type	multiple	multiple
Number of calibrators	-	-
Calibration curve	-	-
OPTIONS		
Blank absorbance limit	0.200	0.200
Kinetic blank limit	-	-
Linearity limit	1000	1000
Substrate depletion	-	-

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