Colestat

enzimático AA

Enzymatic method for the determination of cholesterol in serum or plasma

SUMMARY

Isolated cholesterol determination has a limited diagnostic value. However, its concentration varies in a predictable way in a large number of clinical conditions. Cholesterol is one of the factors contributing to the formation of atheroma since atherosclerosis complications are mainly found in hypercholesterolemic individuals.
Several epidemiological studies have shown that, among males over 40 years old, the risk of developing coronary heart disease for individuals with blood cholesterol lower than or equal to 2.10 g/l, is 3 times less for individuals with over 2.30 g/l and 6 times less for individuals with over 2.60 g/l.

PRINCIPLE

The reaction system is as follows:

$$\text{Cholesterol esters} \xrightarrow{\text{CHE}} \text{cholesterol + fatty acids}$$

$$\text{Cholesterol + } O_2 \xrightarrow{\text{CHOD}} \text{cholesten-3-one + } H_2O_2$$

$$H_2O_2 + \text{4-AP + acceptor} \xrightarrow{\text{POD}} \text{quinoneimine + } H_2O$$

PROVIDED REAGENTS

S. Standard: 2 g/l cholesterol solution. See PROCEDURE LIMITATIONS.
A. Reagent A: vials containing cholesterol esterase (CHE), cholesterol oxidase (CHOD), peroxidase (POD) and 4-aminophenazone (4-AP).
B. Reagent B: Good buffer solution pH 6.8 containing phenol and sodium cholate.

Final concentrations

CHE	≥ 100 U/l
CHOD	≥ 100 U/l
POD	≥ 1000 U/l
4-AP	0.2 mmol/l
Good	50 mmol/l; pH 6.8
Phenol	15 mmol/l
Sodium cholate	0.2 mmol/l

NON-PROVIDED REAGENTS

Wiener lab.'s **Calibrador A plus**.

INSTRUCTIONS FOR USE

Working Reagent: dissolve the content of a Reagent A vial with a part of Reagent B. Transfer to the Reagent B bottle, rinsing the vial several times with it. Mix until complete dissolution. Homogenize and date.

WARNINGS

Reagents are for "in vitro" diagnostic use.
Use the reagents according to the working procedures for clinical laboratories.
The reagents and samples should be discarded according to the local regulations in force.

STABILITY AND STORAGE INSTRUCTIONS

Provided Reagents: stable in refrigerator (2-10ºC) until the expiration date shown on the box.
Working Reagent: stable for 60 days in refrigerator (2-10ºC) since preparation.

INSTABILITY OR DETERIORATION OF REAGENTS

Blank readings over 0.160 O.D. indicate reagent deterioration. Discard in such case.

SAMPLE

Serum or plasma
a) Collection: obtain in the usual way.
b) Additives: it is recommended to use only heparin as anticoagulant to obtain plasma.
c) Known interfering substances:
- Ordinary anticoagulants, except heparin, interfere with the test.
- Do not use sera with visible or intense hemolysis since they lead to falsely increased results.
- No interferences are observed from bilirubin up to 80 mg/l, ascorbic acid up to 75 mg/l, uric acid up to 200 mg/l, mild hemolysis.

See Young, D.S. in References for effect of drugs on the present method.
d) Stability and storage instructions: without preservatives, serum cholesterol is stable for at least one week in refrigerator and 2 months in a freezer.

REQUIRED MATERIAL (non-provided)

- Spectrophotometer or photocolorimeter
- Micropipettes and pipettes for measuring the stated volumes
- Tubes or spectrophotometric cuvettes.
- Water bath at 37ºC.
- Stopwatch.

ASSAY CONDITIONS

- Wavelength: 505 nm in spectrophotometer or in photocolorimeter with green filter (490-530 nm).
- Reaction temperature: 37ºC
- Reaction time: 5 minutes

- Sample volume: 10 ul
- Working Reagent volume: 1 ml
- Final reaction volume: 1.01 ml

Sample and Reagent volumes may be proportionally increased or decreased (e.g. 20 ul Sample + 2 ml Working Reagent or 50 ul + 5 ml).

PROCEDURE

In three colorimeter test tubes or spectrophotometer cuvettes labeled B (Blank), S (Standard) and U (Unknown), place:

	B	S	U
Standard	-	10 ul	-
Sample	-	-	10 ul
Working Reagent	1 ml	1 ml	1 ml

Incubate in water bath at 37°C for 5 minutes or for 20 minutes at room temperature (25°C). Read in spectrophotometer at 505 nm or in photocolorimeter with green filter (490-530 nm), setting instrument to zero with the Blank.

STABILITY OF FINAL REACTION

Final reaction color is stable for 30 minutes, thus absorbance should be read within that period.

CALCULATIONS

$$\text{Cholesterol (g/l)} = U \times f \qquad f = \frac{2.00 \text{ g/l}}{S}$$

CONVERSION UNITS

Cholesterol (g/l) = cholesterol (mg/dl) x 0.01
Cholesterol (mmol/l) = cholesterol (g/l) x 2.59
Cholesterol (g/l) = cholesterol (mmol/l) x 0.39

QUALITY CONTROL METHOD

Each time the test is performed, analyze two levels of a quality control material **(Standatrol S-E 2 niveles**) with known cholesterol concentration.

REFERENCE VALUES

The National Cholesterol Education Program (NCEP) Expert Panel provided the following reference values:

Desirable: < 2.00 g/l
Borderline high: 2.00 - 2.39 g/l
High: ≥ 2.40 g/l

It is recommended that each laboratory establishes its own intervals and reference values

PROCEDURE LIMITATIONS

See Known interfering substances under SAMPLE.
Reducing agents weaken color response, while oxidants color the Reagent increasing the Blanks.
Detergents, heavy metals and cyanides are enzyme inhibitors.
Do not use the Standard in autoanalyzers due to the different surface tension in relation to the serum, caused by the solvent used in its preparation.

PERFORMANCE

a) Reproducibility: when replicates of the same sample were simultaneously assayed in 10 different days, the following values were obtained:

Level	S.D.	C.V.
1.24 g/l	± 0.043 g/l	3.49 %
3.31 g/l	± 0.115 g/l	3.48 %

b) Recovery: by adding known amounts of cholesterol to different sera, it was observed that recovery ranged between 98 and 101% for every level of cholesterol between 1.90 and 4.79 g/l.

c) Detection limit: depends on the photometer used. For a 0.001 O.D. reading, the minimum visible change of concentration will be of approximately 0.0063 g/l.

d) Linearity: reaction is linear up to 5 g/l, for higher values dilute 1:2 with Blank and repeat the reading multiplying the final result by 2.

PARAMETERS FOR AUTOANALYZERS

For programming instructions check the user manual of the autoanalyzer in use. For calibration use Wiener lab's **Calibra-dor A plus**, following the autoanalyzer requirements.

WIENER LAB. PROVIDES

- 1 x 100 ml (Cat. 1220110).
- 4 x 100 ml (Cat. 1220001).

Colestat *enzimático AA* reagents together with **HDL Colesterol *Reactivo Precipitante***, **HDL Colesterol FT** and **LDL Colesterol *Reactivo Precipitante*** (separately provided by Wiener lab.) can be used to determine High Density Lipoprotein Cholesterol (HDL Cholesterol) and Low Density Lipoprotein Cholesterol (LDL Cholesterol).

REFERENCES

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- Expert Panel of National Cholesterol Education Program - JAMA 285/19:2486 (2001).
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Symbols

The following symbols are used in packaging for Wiener lab. diagnostic reagent kits.

CE — This product fulfills the requirements of the European Directive 98/79 EC for "in vitro" diagnostic medical devices

EC REP — Authorized representative in the European Community

IVD — "In vitro" diagnostic medical device

Σ — Conteins sufficient for <n> tests

Use by

Temperature limitation (store at)

Do not freeze

Biological risks

Volume after reconstitution

Cont. — Contents

LOT — Batch code

Manufactured by:

Xn — Harmful

Corrosive / Caustic

Xi — Irritant

Consult instructions for use

Calibr. — Calibrator

CONTROL — Control

CONTROL + — Positive Control

CONTROL − — Negative Control

REF — Catalog number

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