LIQUID LINE

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 that contributes 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:

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

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

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

PROVIDED REAGENTS

S. Standard*: 2 g/l cholesterol solution.

A. Reagent A: solution containing cholesterol esterase (CHE), cholesterol oxidase (CHOD), peroxidase (POD), 4-amino-phenazone (4-AP) and Good buffer, which contains phenol and sodium cholate, in the following concentrations:

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

NON-PROVIDED REAGENTS

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

INSTRUCTIONS FOR USE

Provided Reagents: ready to use.

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. Do not expose to high temperatures for long periods of time.

INSTABILITY OR DETERIORATION OF REAGENTS

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

SAMPLE

Serum or plasma.

a) Collection: obtain in the usual way.

b) Additives: when using plasma, it is recommended to use only heparin as anticoagulant to obtain it.

c) Known interfering substances:

- With the exception of heparin, common anticoagulants 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 1 week in a 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 490-530 nm in photocolorimeter with green filter.
- Reaction temperature: 37ºC
- Reaction time: 5 minutes
- Sample volume: 10 ul
- Reagent volume: 1 ml
- Final reaction volume: 1.01 ml

Sample and Reagent A volumes may be varied proportionally (e.g. 20 ul Sample + 2 ml Reagent A).

** Non-provided with all kit sizes*

PROCEDURE

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

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

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

cholesterol (g/l)= D x f where $f = \frac{2.00 \text{ g/l}}{S}$

UNITS CONVERSION

cholesterol (g/l) = cholesterol (mg/dl) x 0.01
cholesterol (mmol/l) = cholesterol (g/l) x 2.59
cholesterol (g/l) = cholesterol (mmol/dl) 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 decrease the color response, while oxidants color the Reagent A 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 regards to serum, caused by the solvent used in its preparation.
It is recommended to perform one weekly calibration or each time values outside the acceptable Controls (Standatrol S-E 2 niveles) normal range are obtained.

PERFORMANCE

a) Reproducibility: processing replicates of the same samples in 10 different days, the following results 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: adding known amounts of cholesterol to different sera, for cholesterol levels between 1.90 and 4.79 g/l, a recovery between 98 and 101% was obtained.
c) Detection limit: it depends on the photometer used. For a 0.001 O.D. reading, the minimum detectable change of concentration will be of approximately 0.0063 g/l.
d) Linearity: the 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's manual of the autoanalyzer in use.
For calibration use Wiener lab's **Calibrador A Plus**, following the autoanalyzer requirements.

WIENER LAB. PROVIDES

- 6 x 60 ml (Cat. Nº: 1009308).
- 6 x 60 ml (Cat. Nº: 1009610).
- 4 x 100 ml (Cat. Nº: 1220114).
- 2 x 500 ml (Cat. Nº: 1220222).
- 12 x 50 ml (Cat. Nº 1009253).

Using **Colestat enzimático AA líquida** along with **HDL-Colesterol Reactivo Precipitante**, **HDL Colesterol FT** and **LDL-Colesterol Reactivo Precipitante** (separately provided by Wiener lab.) it is possible to determine the 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).
- Young, D.S. - "Effects of Drugs on Clinical Laboratory Tests", AACC Press, 4th ed., 2001.

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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