

TOTAL CHOLESTEROL

Enzymatic colorimetric determination of cholesterol in serum and plasma

TEST SUMMARY

Esterified cholesterol is hydrolyzed in presence of cholesterol esterase, in cholesterol and fatty acids. The cholesterol formed in this way, with the free cholesterol, is oxidized from cholesterol oxidase to cholesterol-4-ene-3-one and hydrogen peroxidase. For peroxidase action, the hydrogen peroxidase reacts with 4-aminoantipyrine and p-chlorophenol, forming a red compound which intensity is proportional to the cholesterol concentration in the sample.

SAMPLES

Serum, plasma EDTA.
Stability: 3 days at 2-8°C and 1 month at -20°C.

REAGENTS

Sole Reagent: Good's buffer 50 mM pH 7.20, sodium colate 8 mM, CHE ≥ 400 U/L, CHOD ≥ 200 U/L, POD ≥ 500 U/L, 4-AAP 0.6 mM, 4-chlorophenol 2 mM.

Standard: Cholesterol 200 mg/dl.

MATERIALS REQUIRED BUT NOT SUPPLIED

Current laboratory instrumentation. Spectrophotometer UV/VIS with thermostatic cuvette holder. Automatic micropipettes. Glass or high quality polystyrene cuvettes. Physiological solution.

PRECAUTIONS

Reagent may contain some non-reactive and preservative components. It is suggested to handle carefully it, avoiding contact with skin and swallow. Perform the test according to the general "Good Laboratory Practice" (GPL) guidelines.

REAGENTS PREPARATION

Reagent A and B are supplied in liquid form ready to use.
Stability: until expiration date on label stored at 2-8°C away from light sources.
Stability after first opening: ≥ 60 days at 2-8°C.

PROCEDURE

Kind of analysis: End point
Reading time: 5 minutes
Wavelength: 510 nm (480-520)
Temperature: 37°C
Lightpath: 1 cm
Zero: Reagent Blank

Reagents	Blank	Standard	Sample
Distilled water	10 µl	--	--
Standard	--	10 µl	--
Sample	--	--	10 µl
Sole Reagent	1 ml	1 ml	1 ml

CALCULATION

Serum/Plasma Cholesterol (mg/dl)

(A sample/A standard) x 200

EXPECTED VALUES

Desirable: 140 – 200 mg/dl
Borderline/high risk: 200 – 240 mg/dl
High risk: > 240 mg/dl

Each laboratory should establish appropriate reference intervals related to its population.

NOTES

- If the results are incompatible with clinical presentation, they have to be evaluated within a total clinical study.
- Only for IVD use.

CALIBRATION/QUALITY CONTROL

It is suggested to perform an internal quality control. For this purpose the following control sera on human base are available on request:

QN 0050 CH 10 x 5 ml

Control Sera normal values

QP 0050 CH 10 x 5 ml

Control Sera pathological values

TEST PERFORMANCE

Precision

Intra-assay (n = 25)	Media (mg/dl)	SD (mg/dl)	CV%
Sample 1	199.84	1.8636	0.93
Sample 2	292.6	1.8484	0.63

Inter-assay (n = 25)	Media (mg/dl)	SD (mg/dl)	CV%
Sample 1	201.04	2.2263	1.11
Sample 2	292.96	2.424	0.83

Sensitivity/limit of detection

Method is able to discriminate up to 1 mg/dl.

Linearity

Method is linear up to 700 mg/dl.
If the value is exceeded, is suggested to dilute sample 1+9 with saline and to repeat the test, multiplying the results by 10.

Methods comparison

A comparison with a commercial available product gave the following results in a comparison on 28 samples:

Total Cholesterol LTA = x
Cholesterol competitor = y
n = 28

$$y = 1,00331x - 1,00447 \quad r = 0,99129$$

Interferences

No interference was observed by the presence of:
hemoglobin ≤ 300 mg/dl
bilirubin ≤ 40 mg/dl
lipids ≤ 850 mg/dl

WASTE DISPOSAL

Product is intended for professional laboratories. Waste products must be handled as per relevant security cards and local regulations.

PACKAGING

CODE CC01000 (400 TESTS)
Sole Reagent 4 x 100 ml (liquid)
Standard 1 x 5 ml (liquid)

REFERENCES

Trinder P., - J.Clin. Path. 22, 158 (1969).
Allain C.C., Poon L.S., Chan C.S., Richmond W., Fu P.C. - Clin. Chem. 20,470 (1974).
National Cholesterol Education Program (NCEP) recommended values for cholesterol.
Tietz Textbook of Clinical Chemistry, Second Edition, Burtis-Ashwood (1994).

MANUFACTURER

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SYMBOLS

- IVD Only for IVD use
- LOT Lot of manufacturing
- REF Code number
- Storage temperature interval
- Expiration date (year, month)
- Warning, read enclosed documents
- Read the directions
- Biological risk

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