Wako

Cholesterol E

For Research Use Only. Not for use in diagnostic procedures.

Intended use

The Cholesterol E is an enzymatic colorimetric method for the quantitative determination of total cholesterol in serum.

Summary and explanation of the test

Early methods for determining cholesterol levels were based on chemical colorimetric methods. Since W. Richmond reported an enzymatic method employing CO (Cholesterol Oxidase) in 1972, a highly specific method, it has been used extensively. Wako has developed the Cholesterol E test kit employing a blue chromophore in a simple, one-step procedure.

Principle of the method

Cholesterol esters in the serum are hydrolyzed to free cholesterol and fatty acids in a reaction catalyzed by cholesterol ester hydrolase. The cholesterol produced and the free cholesterol already present in the serum are oxidized in reaction catalyzed by cholesterol oxidase that generates hydrogen peroxide.
The formed hydrogen peroxide participates in quantitative oxidative condensation between 3,5-Dimethoxy-N-ethyl-N-(2-hydroxy-3-sulfopropyl)-aniline sodium salt (DAOS) and 4-aminoantipyrine in the presence of peroxidase. The product of the reaction is a blue pigment. The total amount of cholesterol in the test sample is determined by measurement of the absorbance of the blue color at 600 nm. Since ascorbic acid in serum is decomposed in a reaction catalyzed by ascorbate oxidase (AOD), it has little influence on the assay.

Reagents

(1) Buffer Solution — 2 × 150 mL
50 mmol/L Good's buffer solution, pH6.1
Store at 2-10℃.

(2) Color Reagent — 2 × for 150 mL
1.6 units/mL Cholesterol ester hydrolase
0.31 units/mL Cholesterol oxidase
5.2 units/mL Peroxidase
0.19 mmol/L 4-Aminoantipyrine
4.4 units/mL Ascorbate oxidase
0.95 mmol/L DAOS
Store at 2-10℃.

(3) Standard Solution — 1 × 10 mL
200 mg/dL Cholesterol
Store at 2-10℃.

Warnings and precautions

(1) For Research Use Only. Not for use in diagnostic procedures.
(2) Not to be used internally in humans or animals.
(3) Do not use the reagents described above for any purpose other than described herein. Performance cannot be guaranteed if the reagents are used for otherprocedures or purposes.
(4) Operate the instrument according to operator's manual under appropriate conditions. Consult the instrument manufacturer for details.
(5) Store the reagents under a specified conditions. Do not use reagents past the expiration date stated on each reagent container label.
(6) Do not use reagents which were frozen in error. Such regents may give false results.
(7) After opening reagents, it is recommended to use them immediately. To store opened reagents, cap the bottles and keep them under specified conditions.
(8) Use Wako's Calibrator for preparation of a calibration curve.
Read the instruction sheet in the package of the calibrator thoroughly before use.
(9) If the reagents come in contact with mouth, eye, or skin, wash off immediately with a large amount of water. Consult a physician if necessary.
(10) Be careful not to cut yourself with the aluminum cap when removing it from the vial.
(11) When discarding the reagents, dispose of them according to local or national regulations.

Physical or chemical indications of instability

The presence of precipitates in the regents or values of control sera outside the manufacturer's acceptable range may be an indication of Reagent instability.

Specimen collection and preparation

(1) Sodium fluoride, an inhibitor of glycolysis, has almost no influence on the measurement of total cholesterol. Anticoagulants such as heparin, oxalate, citrate and EDTA do not affect measurements when they are used in their respective usual quantities. Note that in case of using large amounts of heparin, precipitation may occur in the sample.
(2) Measurements should be made immediately after the serum has been obtained. Alternatively, the serum should be stored frozen (– 20℃ or below) until assayed.
(3) In the case of hemolyzed blood, hemoglobin introduces a positive error of 3 mg of total cholesterol/dL per 200 mg of hemoglobin/dL.
This does not present a problem in the analysis of the usual test sample. In the case of heavily hemolyzed samples however, correction must be made according to the above relationship.
Alternatively, a fresh blood sample must be taken.
(4) Bilirubin (up to 40 mg/dL) has almost no influence on measurements.

Warning/Biohazard

Since all specimens are potentially infectious, they should be handled at the Biosafety Level 2 as recommended for any potentially infectious body fluid in the USA Centers for Disease Control/USA National Institutes of Health manual ''Biosafety in Microbiological and Biomedical Laboratories'', and in accordance with any other local or national regulations relating to the safe handling of such materials.

Procedure

Materials supplied

Refer to the section entitled ''Reagents.''

Materials required but not supplied

Micropipette capable of measuring 20 μL volume
Transfer pipette 2.0 mL
Test tubes
Water bath
Spectrophotometer

Reagent preparation

Color Reagent :
(1) The Color Reagent bottle is under negative pressure. Slowly loosen the rubber stopper to prevent contents of the bottle from spilling.
(2) Dissolve the entire contents of one bottle of color reagent in the total amount of Buffer Solution.

Color Solution may be used for 3 weeks after preparation when stored at 2-10℃.

Test procedure

(1) Pipette 20 μL of each sample, standard and blank (distilled water) into pre-labeled tubes.
(2) Add 2 mL of color reagent.
(3) Mix well and incubate at 37℃ for 5 minutes.
(4) Measure the absorbance of the Sample (A_S) and Standard (A_{Std}) against the Blank at 600 nm.

Note ; When measured with two wavelengths :
λ1 = 600 nm (primary)
λ2 = 700 nm (secondary)

Results

(1) From the calibration curve :
The total cholesterol concentration corresponding to the absorbance of Samples (A_S) is read from the calibration curve prepared in advance.
(2) By calculation :
In the case of a usual serum sample, measure the absorbance of the sample (A_S) and the Standard (A_{Std}) against the Blank and calculate total cholesterol concentration from the following formula :

$$C_{sample} = \frac{A_S}{A_{Std}} \times C_{Std}$$

Where Cstd is the concentration of the standard

Example :
Absorbance of sample A_S = 0.309
Concentratin of the standard = 200 mg/dL

Absorbance of the Standard A_{Std} = 0.380
Total cholesterol concentration in the sample (mg/dL)

$$= \frac{0.309}{0.380} \times 200 = 163 \text{ (mg/dL)}$$

Quality control

A quality control program is recommended for all laboratories.
The analysis of control material in both the low and high ranges with each assay is recommended for monitoring the procedure's performance. The values obtained for controls should fall within the manufacturer's acceptable ranges. If values are to be established for unassayed control material, the laboratory should assay each level of control material a sufficient number of times to generate a valid mean and acceptable range.

Performance characteristics

Measurable range

Up to 1000 mg/dL total cholesterol.
(in the case of using the standard procedure)

Calibration

(1) Prepare a diluted standard by adding distilled or deionized water to the Standard Solution as directed below.

Standard Solution	Water	Final Concentration
1.0 mL	1.0 mL	100 mg/dL

(2) Take the diluted standard solution prepared as above (1) and the Standard Solution (undiluted) in the quantities shown below into 4 separate test tubes.

Tube No.	Diluted Stnd. Soln.	Standard Soln. (stock soln.)	Cholesterol Conc. mg/dL
1	0.02 mL	-----	100
2		0.02 mL	200
3		0.04 mL	397.4 (note)
4		0.06 mL	592.2 (note)

Note : The volume of the sample to be taken is 0.02 mL as a rule. However, since 0.04 mL and 0.06 mL were taken in these cases the total volume is increased slightly, the cholesterol values indicated are corrected accordingly.

(3) Process the tubes as directed in the test procedure. To obtain a calibration curve, plot a graph of absorbance along the ordinate against total cholesterol concentration along the abscissa.

Precautions in measurement

(1) The color development is complete in about 2 minutes. However, no change in absorbance occurs if incubation is continued for another 30 minutes. The blue color is stable for about 2 hours, once developed.
(2) Uric acid does not influence measurements. Ascorbic acid has almost no influence on measurements.
(3) Tube made from materials such as polyvinyl chloride, polyethylene and silicone can be used for the color solution.

References

(1) Richmond, W.: Sand. J. Clin. Lab. Invest., 20 (Supple), 126 (1972).
(2) Richmond, W.: Clin. Chem., **19**, 1350 (1973).
(3) Allain, C. C., Poon, L.S., Chan, C.S.G., Richmond, W. Fu, P.C.: Clin. Chem., **20**, 470 (1974).

Ordering information

Code No.	Product	Package
999-02601	Cholesterol E •Buffer Solution 2 × 150 mL •Color Reagent 2 × for 150 mL •Standard Solution 1 × 10 mL	100 Tests

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999-02601F

Manufactured by
Wako Pure Chemical Industries, Ltd.
1-2 Doshomachi 3-Chome, Chuo-Ku Osaka 540-8605 Japan
Tel : +81-6-6203-3749
Fax : +81-6-6203-1917
www.wako-chem.co.jp

Distributed by
Wako Diagnostics
Wako Life Sciences, Inc.
1025 Terra Bella Ave., Suite A
Mountain View, CA 94043 USA
Tel : 877-714-1924
Fax : 804-271-0449
www.wakodiagnostics.com

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