HƯỚNG DẪN SỬ DỤNG TIẾNG ANH

Tài liệu được xác nhận bằng chữ ký số

Hà Nội, ngày 26 t háng 7 năm 2022

Người đại diện hợp pháp của cơ sở

GIÁM ĐỐC

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COD 11585 1 x 80 mL
STORE AT 2-8ºC
Reagents for measurement of LDL cholesterol concentration Only for *in vitro* use in the clinical laboratory

CHOLESTEROL LDL DIRECT

CE

BioSystems REAGENTS & INSTRUMENTS

CHOLESTEROL LDL DIRECT
DETERGENT

PRINCIPLE OF THE METHOD

A specific detergent solubilizes the cholesterol from high density lipoproteins (HDL), very low density lipoproteins (VLDL) and chylomicrons. The cholesterol esters are broken down by cholesterol esterase and cholesterol oxidase in a non-color forming reaction. The second detergent, present in the reagent B, solubilizes cholesterol from low density lipoproteins (LDL) in the sample. The LDL cholesterol is then spectrophotometrically measured by means of the coupled reactions described below[1].

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

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

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

CONTENTS AND COMPOSITION

A. Reagent. 1 x 60 mL. MES buffer > 30 mmol/L, cholesterol esterase < 1.5 U/mL, cholesterol oxidase < 1.5 U/mL, 4-aminoantipyrine 0.5 mmol/L, ascorbate oxidase < 3.0 U/L, peroxidase > 1 U/mL, detergent, pH 6.3.

B. Reagent. 1 x 20 mL. MES buffer > 30 mmol/L, N,N-bis(4-sulfobutyl)-m-toluidine (DSBmT) 1 mmol/L, detergent, pH 6.3.

STORAGE

Store at 2-8ºC.

Reagents are stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use.

Indications of deterioration: Presence of particulate material, turbidity.

AUXILIARY REAGENTS

Biochemistry Calibrator Human (BioSystems cod. 18044) or Cholesterol HDL/LDL Calibrator (BioSystems cod. 11693).

S. Cholesterol HDL/LDL calibrator (cod. 11693). Human serum. Concentration is given on the label. Reconstitute with 1.0 mL of distilled water. Stable for 1 week at 2-8ºC or for 2 months at –18ºC when frozen in aliquots. The concentration value is traceable to the CDC Reference Measurement Procedure (Centers for Disease Control and Prevention).

Components from human origin have been tested and found to be negative for the presence of antibodies anti-HIV and anti-HCV, as well as for Hbs antigen. However, they should be handled cautiously as potentially infectious.

REAGENT PREPARATION

Reagents are provided ready to use.

ADDITIONAL EQUIPMENT

– Thermostatic water bath at 37ºC

– Analyzer, spectrophotometer or photometer with cell holder thermostatable at 37ºC and able to read at (main wavelength) 546 ± 20 nm and (sub-wavelength) 700 nm ± 50 nm.

SAMPLES

Serum, EDTA-treated plasma or sodium heparinized plasma collected by standard procedures.

LDL cholesterol in serum is stable for 5 days at 2-8ºC.

PROCEDURE

1. Bring the Reagents and the photometer to 37ºC.
2. Pipette into a cuvette: (Notes 1 and 2)

Reagent A	750 µL
Serum/Calibrator	7 µL

3. Mix and insert the cuvette into the photometer. Start the stopwatch. After 3-5 minutes, read the absorbance (A_1) at 546/700 nm against distilled water.
4. Pipette into a cuvette:

Reagent B	250 µL

Mix.

5. After 5 minutes, read the absorbance (A_2) at 546/700 nm.

CALCULATIONS

The cholesterol LDL concentration is calculated using the following general formula:

$$\frac{(A_2 - A_1)_{\text{Sample}}}{(A_2 - A_1)_{\text{Calibrator}}} \times C_{\text{Calibrator}} = C_{\text{Sample}}$$

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[2].

Up to 100 mg/dL = 2.59 mmol/L	Optimal
100-129 mg/dL = 2.59-3.34 mmol/L	Near optimal/above optimal
130-159 mg/dL = 3.37-4.12 mmol/L	Borderline High
160-189 mg/dL = 4.14 –4.90-mmol/L	High
> 190 mg/dL = 4.92 mmol/L	Very High

QUALITY CONTROL

It is recommended to use the Lipid Control Serum level I (cod. 18040) and II (cod. 18041) or the Biochemistry Control Serum Human level I (cod. 18042) and II (cod. 18043) to verify the performance of the measurement procedure.

Each laboratory should establish its own internal Quality Control scheme and procedures for corrective action if controls do not recover within the acceptable tolerances.

METROLOGICAL CHARACTERISTICS

– Detection limit: 0.28 mg/dL = 0.007 mmol/L.

– Linearity limit: 990 mg/dL = 25.6 mmol/L.

– Repeatibility (within run):

Mean Concentration	CV	n
146 mg/dL = 3.78 mmol/L	0.7 %	20
210 mg/dL = 5.43 mmol/L	0.6 %	20

– Reproducibility (run to run):

Mean Concentration	CV	n
143 mg/dL = 3.70 mmol/L	2.0 %	40
207 mg/dL = 5.35 mmol/L	1.7 %	40

– 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.

– Interferences: Lipemia (triglycerides 12.9 g/L), hemoglobin (60 g/L) and bilirubin (20 mg/dL) do not interfere. Other drugs and substances may interfere[3].

These metrological characteristics have been obtained using an analyzer. Results may vary if a different instrument or a manual procedure are used.

DIAGNOSTIC CHARACTERISTICS

LDL is the main lipoprotein transporting cholesterol from liver to tissues.

Increased plasma LDL-cholesterol concentrations are positively correlated with the incidence of atherosclerosic diseases, basis of myocardial infarction and cerebrovascular accidents[4,5].

There are several disease states or environmental influences associated with increased levels of LDL-cholesterol: nephrosis, diabetes, obesity, some drugs and smoking[4,5].

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

NOTES

1. Sample and Reagent volumes may be varied as long as the same ratio is maintained.
2. These reagents may be used in several automatic analysers. Instructions for many of them are available on request.

BIBLIOGRAPHY

1. Nauck M, Warnick GR, Rifai N. Methods for measurement of LDL-cholesterol: a critical assessment of direct measurement by homogeneous assays versus calculation. *Clin Chem* 2002; 48: 236-54.
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3. Young DS. Effects of drugs on clinical laboratory tests, 5th ed. AACC Press, 2000.
4. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, 4th ed. Burtis CA, Ashwood ER, Bruns DE. WB Saunders Co, 2005.
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