DIRECT LDL CHOLESTEROL LR

Liquid reagent

CE IVD for in vitro diagnostic use

Ref 10175 - 30+10 ml

Enzymatic direct determination of LDL cholesterol in serum.

INDICATION

Low Density Lipoproteins (LDL) are synthesized in the liver by the action of various lipolyc enzymes on triglyceride-rich Very Low Density Lipoproteins (VLDLs). Specific LDL receptors exist to facilitate the elimination of LDL from plasma by liver parenchimal cells. It has been shown that most of the cholesterol stored in atherosclerotic plaques originates from LDL. For this reason the LDL-Cholesterol concentration is considered to be the most important clinical predictor, of all single parameters, with respect to coronary atherosclerosis. Accurate measurement of LDL-Cholesterol is of vital importance in therapies which focus on lipid reduction to prevent atherosclerosis or reduce its progress and to avoid plaque rupture.

METHOD PRINCIPLE

LDL, VLDL and chylomicrons (CM) bind to PVS and PEGME present in Reagent A forming a (LDL+VLDL+CM)PVS/PEGME complex. Only HDL cholesterol reacts with the enzymes CHOD and CHER. Addition of Reagent B, containing a specific detergent, release LDL from the PVS/PEGME complex. The released LDL reacts with the enzymes to produce H_2O_2 which is quantified by the Trinder reaction.

$$\text{HDL} + \text{LDL} + \text{VLDL} + \text{CM} \xrightarrow{\text{PVS/PEGME}} \text{HDL} + (\text{LDL+VLDL+CM})\text{PVS/PEGME}$$

$$\text{HDL} \xrightarrow{\text{CHOD + CHER}} \text{Fatty acids} + H_2O_2$$

$$(\text{LDL+VLDL+CM})\text{PVS/PEGME} \xrightarrow{\text{Detergent}} \text{LDL}$$

$$\text{LDL} \xrightarrow{\text{CHOD + CHER}} \text{Fatty acids} + H_2O_2$$

$$2H_2O_2 + \text{4-AA} + \text{TODB} \xrightarrow{\text{POD}} 5H_2O + \text{quinone dye}$$

The quantity of quinone dye is proportional to the LDL cholesterol present in the sample.

PVS = Polyvinyl sulfonic acid
PEGME = Polyethylene-glycol-methyl ester
CHOD = Cholesterol oxidase
CHER = Cholesterol esterase
POD = Peroxidase
AA = 4-aminoantipyrine
TODB = N, N-Bis(4-sulfobutyl)-3-methylaniline

COMPOSITION

REAGENT A:	
MES buffer, pH 6.5	50 mmol/l
PVS	5 mmol/l
PEGME	0.05 mmol/l
$MgCl_2$	1 mmol/l
EDTA	1 mmol/l
4-AA	5 mmol/l
CHER	5 KU/l
CHOD	3 KU/l
POD	10 KU/l
Detergent	0.02%

REAGENT B:	
MES buffer, pH 6.5	50 mmol/l
EDTA	1 mmol/l
TODB	2 mmol/l
Detergent	0.02%

Preparation of reagents

The reagents are liquids ready to use.

Storage and stability

Store at 2-8 °C. Do not freeze the reagents! The reagents are stable up to the expiry date stated on the label, if contamination and evaporation are avoided, protected from light. The above conditions are valid if the vials are opened just only for the time to take the reagent, closed immediately with their cap and stored at the indicated conservation temperature.

ANCILLARY EQUIPMENT

- Automatic pipettes
- Photometer
- Analysis cuvettes (optical path = 1 cm)
- Temperature controlled water bath
- NaCl solution 9 g/l
- HDL/LDL Calibrator (Ref. 20120)

SAMPLES

Use fresh fasting patient serum and plasma sample (EDTA, Citrate).

Specimen collection / Preanalytical factors

It is recommended that specimen collection should be carried out in accordance with NCCLS Document H11-A3.

INTERNAL QUALITY CONTROL

It is recommended to use commercial control sera with known LDL cholesterol values. Check that the values obtained are within the reference range provided.

ANALYTICAL PROCEDURE

Working temperature	37 °C
Wavelength	600 nm
Optical path	1 cm
Reaction	End point

Allow the reagents to reach working temperature before using.

Pipette into disposable or well clean cuvettes:

	Blank	Calibrator	Sample
Reagent A	300 µl	300 µl	300 µl
Distilled H_2O	4 µl	-	-
Calibrator	-	4 µl	-
Sample	-	-	4 µl
Mix, incubate 5 minutes. Read absorbance (A_1) of calibrator and samples against Blank. Then add:			
Reagent B	100 µl	100 µl	100 µl
Mix and incubate for 5 minutes. Read absorbance (A_2) of calibrator and samples against Blank.			

Note

- Reaction volumes can be proportionally changed.
- For values upper than 250 mg/dl dilute samples with saline solution and multiply result by the dilution factor.

CALCULATION OF RESULTS

$$\text{LDL Cholesterol, mg/dl} = \frac{(A_2\text{-}A_1)\,\text{sample}}{(A_2\text{-}A_1)\,\text{calibrator}} \times \text{mg/dl calibrator}$$

Conversion factor

LDL Cholesterol [mg/dl] x 0.02586 = LDL Cholesterol [mmol/l]

REFERENCE VALUES

The expected values are the following:

Optimal:	< 100 mg/dl
Near optimal/above optimal:	100÷129 mg/dl
Borderline high:	130÷159 mg/dl
High:	160÷189 mg/dl
Very high:	≥ 190 mg/dl

Each laboratory should establish reference ranges for its own patients population.

ANALYTICAL PERFORMANCES

Precision

Within-run and between-run coefficients of variation have been calculated on replicates of three samples at different LDL cholesterol concentration. The obtained results are reported in the following tables:

Within-run				
Sample	n	Mean (mg/dl)	SD	%CV
Serum 1	80	97.14	1.00	1.0
Serum 2	80	147.37	1.19	0.8
Serum 3	80	211.47	1.38	0.7

Between-run				
Sample	n	Media (mg/dl)	DS	%CV
Serum 1	80	97.14	1.55	1.6
Serum 2	80	147.37	2.23	1.5
Serum 3	80	211.47	2.98	1.4

Linearity

The assay is linear up to 250 mg/dl.

Sensitivity

Test sensitivity, in terms of limit of detection, is 2 mg/dl.

Correlation

A correlation study comparing the present method an a commercial one gave the following results:

$y = 1.0883x + 0.6078$ mg/dl $\quad r = 0.9804$

Interferences

Bilirubin	> 40 mg/dl
Triglycerides	> 1000 mg/dl
Ascorbic acid	> 10 mmol/l
Hemoglobin	> 1000 mg/dl

PRECAUTIONS IN USE

The reagents contain inactive components such as preservatives (Sodium azide or others), surfactants etc. The total concentration of these components is lower than the limits reported by 67/548/EEC and 88/379/EEC directives about classification, packaging and labelling of dangerous substances. However, the reagents should be handled with caution, avoiding swallowing and contact with skin, eyes and mucous membranes. The use of laboratory reagents according to good laboratory practice is recommended.

Waste Management

Please refer to local legal requirements.

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