LDL-DIRECT

Direct Method

DIATEK

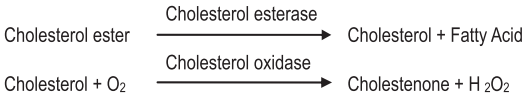
CLINICAL SIGNIFICANCE

Low density lipoproteins (LDL) are synthesized in the liver by the action of various lipolytic enzymes on triglyceride rich very-low-density lipoproteins (VLDLs). Specific LDL receptors exist to facilitate the elimination of LDL from plasma by liver parenchymal 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.
In this diagnostic test kit an elimination method for the measurement of LDL-Cholesterol, without sample pretreatment, is presented which correlates well with precipitation and ultracentrifugation methods.

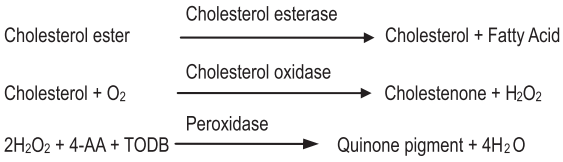
ASSAY PRINCIPLE

The assay consists of 2 distinct reaction steps:

1. Elimination of chylomicron, VLDL-Cholesterol and HDL-Cholesterol by cholesterol esterase, cholesterol oxidase.



2. Specific measurement of LDL-Cholesterol after release of LDL-Cholesterol by detergents in Reagent 2.



The intensity of the quinoneimine dye produced is directly proportional to the cholesterol concentration when measured at 600 nm.

SAMPLE COLLECTION AND PREPARATION

Samples may be taken from non-fasting or fasting individuals. Serum, heparinized plasma are the recommended samples. Serum samples are stable for 6 days at 2-8℃ or 1 year when stored at -70℃.
EDTA plasma causes decreased values.

REAGENT COMPOSITION

Pack Size :	40 mL	80 mL	100 mL	160 mL	320 mL
Reagent 1 (R1)	**30 mL**	**60 mL**	**75 mL**	**2x60 mL**	**3x80 mL**
Cholesterol esterase	5 KU				
Cholesterol oxidase	5 KU				
Peroxidase	20 KU				
4-aminoantipyrine	0.5 g/l				
$MgCl_2$	2 mmol/l				
Detergent	0.5 g/l				
Preservative	0.5 g/l				
Goods buffer	10 mmol/l				
Reagent 2 (R2)	**10 mL**	**20 mL**	**25 mL**	**2x20 mL**	**2x40 mL**
TODB	2 mmol/l				
Detergent	1%				
Preservative	0.5 g/l				
Goods buffer	10 mmol/l				

Calibrator : Reconstitute with 1.0 ml D. Water
Stable for 7 days when strictly stored at 2-8°C.
Calibrator Conc : See on the vial

STABILITY AND PREPARATION OF REAGENTS

All reagents are ready to use.
Stable up to the expiry date when stored at 2-8°C.
Once opened the reagent is stable for 1 month

MATERIALS REQUIRED BUT NOT PROVIDED :

LDL Cholesterol Control (Use of assayed QC sera is recommended to validate test result).

ASSAY CONDITIONS:	***Clear sample***	***Lipemic or turbid sample***
Wavelength : (570-620 nm)	600 nm	600 nm
Cuvette light path:	1 cm	1 cm
Constant temperature :	37ºC	37ºC
Reaction (Mode) :	End Point	Fixed Time
Calibrator Conc :	See on the vial	See on the vial
Delay :	5 sec	5 sec
Read Time :	-	180 sec
Linearity :	690 mg/dL	690 mg/dL
Unit :	mg/dL	mg/dL
Blank :	Reagent	D. Water
Slope of reaction :	Increasing	Increasing

ASSAY PROCEDURE :

	B	C	T
Reagent (1)	450µl	450µl	450µl
LDL Calibrator		6µl	
Specimen			6µl
Mix and incubate for 5 minutes at 37°C			
Reagent (2)	150µl	150µl	150µl

END POINT METHOD
Mix and incubate for 5 minutes at 37°C. Mix and read absorbance Calibrator (C) and Test (T) against Blank (B) at 600nm

FIXED TIME
Mix and read absorbance of Calibrator (C) and Test (T) against D. Water Blank at 600nm with delay time 5 sec and read time 180 sec

CALCULATION (END POINT)

$$\text{LDL-C Conc.} = \frac{A_{Test} - A_{Blank}}{A_{Calibrator} - A_{Blank}} \times \text{Calibrator Conc.}$$

CALCULATION (FIXED TIME)

$$\text{LDL-C Conc.} = \frac{\Delta A_{Test}}{\Delta A_{Calibrator}} \times \text{Calibrator Conc.}$$

NORMAL VALUE

Adult levels ≤ 129 mg/dl
It is recommended that each laboratories should establish its own reference range to reflect age sex diet and geographical location and population

LINEARITY

The method is linear up to 690 mg/dl. Sample above this concentration should be diluted with 0.9% NaCl and reassay. Multiply the result by dilution factor.

SPECIFIC PERFORMANCE CHARACTERISTICS INTERFERENCES

The following analytes were tested up to the levels indicated and found not to interfere Ascorbic acid 50 mg/dl, Bilirubin 20mg/dl , Hemoglobin 500mg/dl, Intralipid : 500mg/dl

SENSITIVITY

The sensitivity of the assay is 5 mg/dl

REFERENCES

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