ANA BIO CLR LDL CHOLESTEROL

(Homogenous direct Method)

Intended Use

LDL Cholesterol is a reagent kit used for the determination of LDL-cholesterol based on enzymatic homogenous method.

Principle

The LDL cholesterol reagent is produced using a combination of detergents and phosphorus compounds which specifically bind HDL, VLDL and CM (chilomicrons) but not LDL. This combination impedes HDL, VLDL and CM from reacting with CO (cholesterol oxidase) and CE (cholesterol esterase), while LDL-cholesterol is able to react with both enzymes.

$$\text{LDL (Cholesterol esters)} + H_2O \xrightarrow{CE} \text{Cholesterol} + \text{Fatty Acid}$$

$$\text{Free cholesterol} + O_2 \xrightarrow{CO} \text{Delta}_4\text{ –cholestenon} + H_2O_2$$

$$2H_2O_2 + \text{4-AA} + \text{HDAOS} \xrightarrow{POD} 4H_2O + \text{Quinone dye}$$

The compound (Quinone dye) which forms is read at λ 546 nm, develops a color, the intensity of which is proportional to the LDL concentration in the test sample.

Reagent provided

1. Reagent – R1
2. Reagent – R2
3. Calibrator (Con. As on vial)

Reagent storage and stability

The kit should be stored at 2° - 8℃ and is stable till the expiry date indicated on the label. DO NOT FREEZE THE REAGENT.

Reagent Preparation

Liquid reagents ready for use. After opening the reagents of R1 and R2 are stable for 60 days if closed, stored at 2° - 8℃, and protect from direct light. Do not mix different batches.

Specimen collection and preservation

Serum or heparinized plasma samples should be used. Samples can be stored for 7 days at 4-8 ℃ and 30 days at -20 ℃.

Assay guidelines for Analyzers

Reaction Type	End Point (2 step)
Reaction time	5 + 5 mins
Wave length	546 nm.
Flow cell temperature	37˚C
Blank	Reagent
Sample volume	6 µl (0.006 ml)
Reagent Volume	0.600 ml + 0.200 ml
Linearity	Up to 450 mg/dl

Perform the assay as given below:

	Blank	**Calibrator**	**Sample**
	-	6 µl (0.006 ml)	6 µl (0.006 ml)
R1	0.600 ml	0.600 ml	0.600 ml
Mix and incubate for 5 minutes at 37℃.			
R2	0.200 ml	0.200 ml	0.200 ml
Mix and incubate for 5 minutes at 37℃. Measure the absorbance at 546 nm.			

Calculation

$$\text{LDL-Cholesterol (mg/dl)} = \frac{\text{Abs. of Sample}}{\text{Abs. of Calibrator}} \times \text{Conc. of Calibrator}$$

Normal range
Serum/Plasma.
Men and Women:

- Normal values (no risk): <130 mg/dl (<3.37 mmol/L)
- Borderline (moderate risk): 130-159 mg/dl (3.37 - 4.12 mmol/L)
- High value (high risk): >160 mg/dl (>4.13 mmol/L)

Note: Expected range varies from population to population and each laboratory should establish its own normal range.

Limitation
Reaction is linear up to 450 mg/dl. If the LDL cholesterol value exceeds 450 mg/dl, then dilute the specimen with normal saline and repeat the assay. In such case the results obtained should be multiplied by dilution factor to obtain correct LDL cholesterol value.

Quality Control
To ensure adequate quality control measures, it is recommended that each batch should include a normal and an abnormal commercial reference control serum. It should be realized that the use of qualitycontrol material checks both instrument and reagent functions together. Factors which might affect the performance of this test include proper instrument function, temperature control, cleanliness of glassware, Wavelength setting and Expiration date of reagents.

Accuracy-Recovery
The recovery of LDL Cholesterol from samples at known concentrations showed an accuracy of 100%.

Interference
The high dilution of the sample with the reagent reduces to a minimum the interference by lipids. Bilirubin below 40 mg/dl does not interfere in the reaction. Haemoglobin interferes at concentrations above 500 mg/dl and Ascorbic Acid in concentrations over 100 mg/dl does not cause interference.

Sensitivity
At 546 nm a concentration of 3.45mg/dl of LDL Cholesterol can estimate.

References

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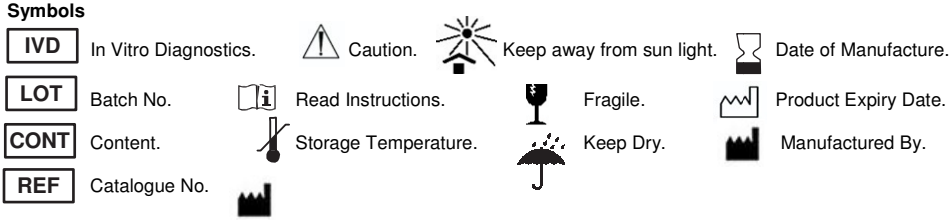
2. Thomas, L. (ed.), Clinical Laboratory Diagnostics; Use and Assessment of Clinical Laboratory Results, 1st edition, TH-Books Verlagsgesellachaft mbH, Franckufurt Main, Germany 1998, pp. 172.
3. Bachorik, Paul S. and Ross, John W., National Cholesterol Education Program Recommendations for Measurement of Low- Density Lipoprotein Cholesterol: Executive Summary Clin Chem. 1995;41;1714-1420.
4. Rifai, N. et al., Measurement. of Low-Density Lipoprotein Cholesterol in Serum: a Status report, Clin Chem. 1992;38.
5. Aufenanger, J. and Zawta, B., pre-analytical Aspects of Lipoprotein Measurement., Clin Lab 1999:45.

Symbols



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