ANA BIO CLR HDL CHOLESTEROL

(Homogenous direct Method)

Intended Use

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

Principle

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

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

$$\text{Free cholesterol} + O_2 \xrightarrow{CO} \text{Delta 4 –cholestenon} + H_2O_2$$

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

The compound (Quinone dye) which forms is read at λ 578 nm, develops a color, the intensity of which is proportional to the HDL 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

Blood should be collected in a clean and dry container. Fasting blood is preferred for cholesterol assay. Cholesterol in the serum is stable for 7 days when stored at 2°-8℃ and 60 days when stored at -20℃

Assay guidelines for Analyzers

Reaction Type	End Point (2 step)
Reaction time	5 + 5 mins
Wave length	578 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 150 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 578 nm.			

Calculation

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

Normal range
Serum/Plasma.

	Male	Frmale
Normal values(No risk)	> 55 mg/dl (> 1.45 mmol/L)	> 65 mg/dl (> 1.68 mmol/L)
Borderline (Moderate risk)	35-55 mg/dl (0.90-1.45 mmol/L)	45-65 mg/dl (1.15-1.68 mmol/L)
High value (High risk)	< 35 mg/dl (< 0.90 mmol/L)	< 45 mg/dl (< 1.15 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 150 mg/dl. If the HDL cholesterol value exceeds 150 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 HDL 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 quality control material checks both instrument and reagent functions together. Factors which might affect the performance of this test include proper instrument function, temperature control, and cleanliness of glassware, Wavelength setting and Expiration date of reagents.

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

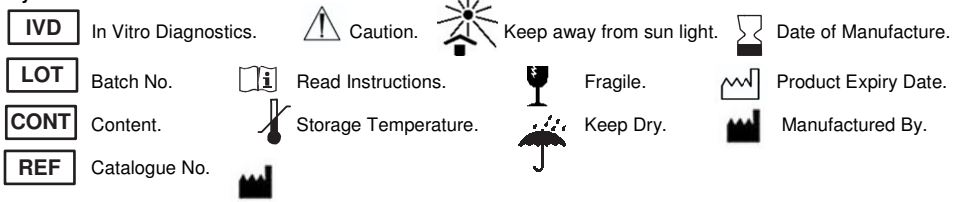
Interference
The high dilution of the sample with the reagent reduces to a minimum the interference by lipids. Bilirubin below 40 mg/dl does not interfere in the reaction. Haemoglobin interferes at concentrations above 500 mg/dl and Ascorbic Acid in concentrations over 100 mg/dl does not cause interference.

Sensitivity
At 578 nm a concentration of 2.39mg/dl of HDL Cholesterol can estimate.

References

1. Butris, CA and Ashwood, E.R (ed), Tietz Fundamentals of Clinical Chemistry , 4th edition, W B Saunders Company, Philadelphia, 1996, p. 376,383-384.
2. Thomas, L. (ed.), Clinical Laboratory Diagnostics; Use and Assessment of Clinical Laboratory Results, 1st edition, TH-Books Verlagsgesellachaft mbH, Franckufurt Main, Germany, pp. 167-171, 1998.
3. Sugiuchi H. et al., Direct Measurement of High-Density Lipoprotein Cholesterol in Serum with polyethylene Glycol-Modified Enzymes and Sulphated a-Cyclodestrin. Clin Chem 1995; 41; 717-723.
4. Thomas L. (ed.), LAbur und diagnose 4th ed. Marbrug: Die Medizinische Verlagsgesellschaft, pp. 208, 1992.
5. Assmann G., At what levels of total, low- or high-density lipoprotein cholesterol should diet/drug therapy be initiated? European guidelines. Amer J Cardiol 1990; 65; 11F.

Symbols



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