ANA BIO ISP HDL CHOLESTEROL

(Homogenous direct Method)

For Miura Instruments

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.

HDL (Cholesterol esters) + H2O $\xrightarrow{CE}$ Cholesterol + Fatty Acid

Free cholesterol + O2 $\xrightarrow{CO}$ Delta 4 –cholestenon + H2O2

2H2O2 + 4-AA + HDAOS $\xrightarrow{POD}$ 4H2O + 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 HDL concentration in the test sample.

Components & Concentration of Reagents

Reagent	Component	Concentration
Reagent 1	Goods Buffer PH 7.0	20 mmol/L
	HDAOS	1 mmol/L
	Stabilizers, excipients & surface active agents	
Reagnet 2	Goods Buffer	20 mmol/L
	4-AAP	3 mmol/L
	CE	≥ 200 U/L
	COD	≥ 3000 U/L
	POD	≥ 1000 U/L
	Stabilizers, excipients & surface active agents	

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℃

Automation
This kit, though developed and manufactured to be used as manual assay and with I.S.E. Miura Analyzer, can be used also with other analyzers able to meet the specifications indicated in section “Reaction conditions – Test procedure” Application sheets are available for automatic instruments.
All applications not explicitly approved by KDPL. Cannot be guaranteed in terms of performance, and must there be established by the operator.

Calibration
For Calibration use the “HDL/LDL Calibrator”

Materials required but not supplied in the kit
Calibrators and controls

Assay guidelines for Analyzer I.S.E. Miura

Analyte Name	HDL Cholesterol
Method Code	HDL
Type	Bichromatic - Substrate start
Unit	mg/dl
Filter F1	546 nm
Filter F2	630 nm
Blank in	Not Used

Step	Reaction Volume	U.M.
Volume reagent R1	150	µl
Sample Volume	2	µl
Incubation R1,S → R2	300	Sec
Volume reagent R2	50	µl
Final Incubation	300	Sec.

Normal range
Serum/Plasma.
Male:
- Normal values (no risk): > 55 mg/dl (> 1.45 mmol/L)
- Borderline (moderate risk): 35-55 mg/dl (0.90-1.45 mmol/L)
- High value (high risk): < 35 mg/dl (< 0.90 mmol/L)

Female:
- Normal values (no risk): > 65 mg/dl (> 1.68 mmol/L)
- Borderline (moderate risk): 45-65 mg/dl (1.15-1.68 mmol/L)
- High value (high risk): < 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 83 mg/dl. If the HDL cholesterol value exceeds 83 mg/dl, then dilute the specimen suitability 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 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, Expiration date of reagents and accuracy of prob aspiration.

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.

Precision of the Method

Within-run					
Range	U.M	Mean	S.D.	C.V. (%)	No. run
Low	mg/dl	36.2	0.44	1.21	20
High	mg/dl	49.2	0.46	1.72	20
Between run					
Range	U.M	Mean	S.D.	C.V. (%)	No. run
Low	mg/dl	36.08	0.35	0.96	60
High	mg/dl	52.96	0.40	1.49	60

Sensitivity
At 546 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

IVD	In Vitro Diagnostics		Caution
LOT	Batch No.		Product Expiry Date
CONT	Content		Manufactured By
	Read Instructions		Date of Manufacture
	Storage Temperature		Keep Dry
REF	Catalogue No.		Fragile
			Keep away from sun light

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