

HDL-C DIRECT

 2 x 100/2 x 37 mL
12011014

Intended Use

 This reagent is intended for *in vitro* quantitative determination of HDL Cholesterol in serum

- Selective Inhibition Method
- Linear up to 150 mg/dL

Clinical Significance

Total blood cholesterol levels have long been known to be related to coronary heart disease (CHD). In recent years, in addition to total cholesterol, high density lipoprotein cholesterol (HDL-C) has become an important tool used to assess an individual risk of developing CHD since a strong negative relationship between HDL-C concentration and the incidence of CHD was reported.

Principle

The reaction between cholesterol other than HDL & enzyme for cholesterol assay is suppressed by the electrostatic interaction between polyanions & cationic substances. Hydrogen peroxide is formed by the free cholesterol in HDL by cholesterol oxidase. Oxidative condensation of EMSE and 4-AA is caused by hydrogen peroxide in the presence of peroxidase, and the absorbance of the resulting red-purple quinone is measured to obtain the cholesterol value in HDL

 Other lipoproteins than HDL $\xrightarrow{\text{Polyanions}}$ Suppress reaction with enzyme
Cationic substances

$$\text{HDL (cholesterol esters)} + \text{H}_2\text{O} \xrightarrow{\text{Cholesterol esterase}} \text{HDL (free cholesterol)} + \text{Free fatty acids}$$

$$\text{HDL (free cholesterol)} + \text{O}_2 + \text{H}^+ \xrightarrow{\text{cholesterol oxidase}} \text{Cholestenone} + \text{H}_2\text{O}_2$$

$$2\text{H}_2\text{O}_2 + 4\text{-AA} + \text{EMSE} + \text{H}_3 + \text{O} \xrightarrow{\text{Peroxidase}} \text{Violet quinone} + 5\text{H}_2\text{O}$$
Kit Components

Reagent/Component	Product Code 12011014	Description
HDL-C Direct R1	2 x 100 mL	N-Ethyl-N-(3-methylphenyl)-N'succinylethyenediame(EMSE)
HDL-C Direct R2	2 x 37 mL	Cholesterol Oxidase 4-Aminoantipyrin(4-AA)

Risk & Safety

Material Safety data sheets (MSDS) will be provided on request

Reagent Preparation

HDL-C Direct R1 & R2 Reagents are ready to use.

Reagent Storage and Stability

The sealed reagents are stable upto the expiry date stated on the label, when stored at 2-8°C, protected from light. Do not freeze.

Open Vial Stability

Once opened the reagents are stable up to 90 days if contamination is avoided.

On-board Calibration Stability

Calibration is stable for 20 days.

Reagent Deterioration

Turbidity or precipitation in any kit component indicates deterioration and the component must be discarded. Values outside the recommended acceptable range for the Agappe Qualicheck Norm & Path control may also be an indication of reagent instability and associated results are invalid. Sample should be retested using fresh vial of reagent.

Precaution

To avoid contamination, use clean laboratory wares. Close reagent bottles immediately after use. Avoid direct exposure of reagent to light. Do not blow into the reagent bottles.

This reagent is only for IVD use and follow the normal precautions required for handling all laboratory reagents.

Waste Management

Reagents must be disposed off in accordance with local regulations.

Sample

Fresh serum (Do not use lipemic or hemolysed sample)

Interferences

No interference for

 Bilirubin up to 40 mg/dL
Haemoglobin up to 500 mg/dL

 Ascorbic acid up to 50 mg/dL
Triglyceride up to 1000 mg/dL

*(when triglyceride in a sample exceeds 1000 mg/dL, dilute the sample 1+9 with saline, repeat the assay and multiply result by 10)

Materials provided

HDL-C Direct R1 & R2 Reagents

Reagents required but not provided

Multicalibrator (Product Code: 11610002), Qualicheck Norm (Product Code: 11601003), Qualicheck Path (Product Code: 11601002)

Unit Conversion

Traditional Unit	SI Unit	Conversion from Traditional to SI
mg/dL	mmol/L	x 0.026

Calibration

Agappe Multicalibrator (Product Code: 11610002) is recommended for calibration of the assay.

Quality control

It is recommended to use Qualicheck Norm (Product Code: 11601003) or Qualicheck Path (Product Code: 11601002) to verify the performance of the measurement procedure. Each Laboratory has to establish its own internal quality control scheme and procedures for corrective action if controls do not recover within the acceptable tolerance.

Reference Range

It is recommended that each laboratory should establish its own reference values.

The following value may be used as guide line.

Male : 35-80 mg/dL

Female : 42- 88 mg/dL

Results obtained for patient samples are to be correlated with clinical findings of patient for interpretation and diagnosis.

Performance
1. Linearity

The reagent is linear up to 150 mg/dL. If the concentration is greater than linearity (150 mg/dL), dilute the sample with normal saline and repeat the assay. Multiply the result with dilution factor.

2. Comparison

 A comparison study has been performed between Agappe reagent and another internationally available reagent yielded a correlation coefficient of r²= 0.9848 and a regression equation of y = 0.9854x.

3. Precision

Control	Intra Run		Inter Run	
	Level 1	Level 2	Level 1	Level 2
n	20	20	20	20
Mean (mg/dL)	67.1	27.1	66.48	27.16
SD	0.87	0.85	1.21	0.55
CV(%)	1.30	3.14	1.82	2.04

Accuracy (mg/dL)

Control	Expected Value	Measured Value
Control Level 1	65 ± 9.8	69.7
Control Level 2	24 ± 6.6	26.6
Qualicheck Norm	30 ± 4.5	32
Qualicheck Path	85 ± 15.5	83.9

4. Sensitivity

Lower detection Limit is 1 mg/dL

Bibliography

- Williams P *et al.*, High density lipoprotein and coronary risk factor, Lancet. 1:72 (1979)
- Gordon, T. Castelli, W.P. Hjortland, M.C. *et al.* Am.J. Med 62, 707-714 (1977)
- Rifai, N. and Warnick, G.R., Ed. Laboratory Measurement of Lipids, Lipoproteins and Apolipoproteins AACC Press. Washington, DC, USA, 1994
- American Heart Association, 2009, NCEP, 2009

SYMBOLS USED ON THE LABELS

IVD IN VITRO DIAGNOSTIC USE SEE PACKAGE INSERT FOR PROCEDURE LOT NUMBER MANUFACTURER'S ADDRESS MANUFACTURING DATE EXPIRY DATE TEMPERATURE LIMIT