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HDL CHOLESTEROL (DIRECT) CE

STABLE LIQUID REAGENT

Cat. No.:	42661,42661S	42662,42662S
	60 ml	500 ml
	(1x45 ml+1x15 ml)	(1x375 ml +1x125 ml)

A direct enzymatic method for the quantitative determination of high density lipoprotein cholesterol (HDL-C) in serum.

Principle
First step.
Cholesterol in LDL, VLDL and chylomicron (CM) in the specimen are catalyzed by Cholesterol esterase and Cholesterol oxidase with addition of R1 solution into the specimen. HDL cholesterol are not catalyzed at first step. The generated hydrogen peroxide is consumed by the condensation reaction with N-ethyl-N-(3.sulfopropyl)-m-anisidine (ESPAS) in existence of Peroxidase

$$\textit{LDL, VLDL and chylomicron} \xrightarrow{\textit{Cholesterol-esterase}} \textit{Free cholesterol+Fatty acid}$$

$$\textit{Free cholesterol} + O_2 \xrightarrow{\textit{Cholesterol-oxidase}} \Delta^4\textit{-cholestenone} + H_2O_2$$

$$H_2O_2 + \textit{ESPAS*} \xrightarrow{\textit{Peroxidase}} \textit{Colourless condensation product}$$

Second step.
Subsequently, HDL cholesterol in the specimen is catalyzed by Cholesterol esterase and Cholesterol oxidase with addition of R2 solution into the specimen. ESPAS and 4-aminoantipyrine (4-AA) are condensed by the generated hydrogen peroxide and Peroxidase to generate a quinone dye. The absorbance of the generated quinone dye is analyzed to obtain the HDL cholesterol concentration in the specimen with reference of a standard solution.

$$\textit{HDL-C} \xrightarrow{\textit{Cholesterol-esterase}} \textit{Free cholesterol + Fatty acid}$$

$$\textit{Free cholesterol} + O_2 \xrightarrow{\textit{Cholesterol-oxidase}} \Delta^4\textit{-cholestenone} + H_2O_2$$

$$2\,H_2O_2 + \textit{4-aminoantipyrine} + \textit{ESPAS*} \xrightarrow{\textit{Peroxidase}} \textit{Quinoneimine dye}$$

Reference values
Adult male: **0.92-2.07 mmol/l (35.3-79.5 mg/dl)**
Adult female: **1.09-2.29 mmol/l (42.0-88.0 mg/dl)**
It is recommended that each laboratory should assign its own normal range.

Reagents
R1
Cholesterol Esterase
Cholesterol Oxidase
Peroxidase
ESPAS
R2
Cholesterol Esterase
4-aminoantipyrine
3. HDL cholesterol standard
Ready for use.For details please check the insert.
Available only in Cat. No.: 42661S and 42662S

Onboard stability: 60 days without calibration

Samples
Use serum as a specimen. It is recommended to measure HDL-C immediately after collection.

PROCEDURE

Reagents 1 and 2 are ready for use.

Assay conditions

Main wavelength:	600 nm
Sub wavelength:	800 nm
Light path:	1 cm
Temperature:	37 °C
Method:	endpoint (increasing)

Pipette into cuvette

	Reagent blank	Standard	Sample
Reagent R1	225 µl	225 µl	225 µl
Standard		2,4 µl	
Sample			2,4 µl
Distilled water	2,4 µl		
Mix and incubate for 5 minutes and then measure the difference between absorbance at 600 nm and 800 nm against the reference			
Absorbance1	A(bl)1	A(std)1	A(s)1
Reagent R2	75 µl	75 µl	75 µl
Mix and incubate for 5 minutes and then measure the difference between absorbance at 600 nm and 800 nm against the reference			
Absorbance2	A(bl)2	A(std)2	A(s)2

Calibration
S1: Distilled water

S2: Sentinel HC calibrator

Wako HC calibrator

Randox HC calibrator

Calibration frequency
Two point calibration is recommended:
- after reagent lot change,
- as required following quality control procedures.

Calculation

$$\text{HDL - C (mg/dl)} = \frac{(A(s)2 - A(s)1) - (A(bl)2 - A(bl)1)}{(A(std)2 - A(std)1) - (A(bl)2 - A(bl)2)} \times \text{std. conc (mg/dl)}$$

Quality control
A quality control program is recommended for all clinical laboratories. The analysis of control material in both the normal and abnormal ranges with each assay is recommended for monitoring the performance of the procedure. Each laboratory should establish corrective measures to be taken if values fall outside the limits

PERFORMANCES DATA

Linearity

The test is linear up to 3,90 mmol/l (150 mg/dl).
The reagent is linear between the concentration of 1-150 mg/dl (0.026-3,90 mmol/l).

Sensitivity
The absorbance when measuring water is less than 0,05. The absorbance when measuring standard (100 mg/dl) is in the range of 0,05-0,17

Specificity
When measuring control serum, the data spread of assay expected value is within 10%
When the sample's triglyceride concentration exceeds up to 1000 mg/dl, dilute the sample with physiological saline and repeat the measurement.
There is minimal interference of measurements with up to 20 mg/dl bilirubin, 50 mg/dl ascorbic acid, 500 mg/dl hemoglobin, 1200 formazine turbidity unit, 200 mg/dl EDTA (Na salt), and 80 U/ml heparin.

Precision

	Intra-run precision
Concentration (mg/dl)	**CV%**
34,8	1,07
97,6	0,90

Correlation
A comparative study has been performed between our reagent and other commercial HDL-cholesterol reagent.The parameters of linear regression are as follows:
y=0,9895x+2,8556 (mg/dl), r=0,9952, N=73

Note
Do not use the pretreatment reagent, which was frozen by mistake.

For *in vitro* diagnostic use only.

The following symbols are used on labels

IVD For in vitro diagnostic use

Use by (last day of the month)

Temperature limitation

LOT Batch Code

REF Code

Bibliography

National Cholesterol Education Program : Executive summary of third report of the National Cholesterol Education Program expert panel on detection, evaluattion and treatment of high blood cholesterol in adults (J AMA,285:2486-2497,2001)