



HDL - Direct



Direct enzymatic method for the quantitative determination of Cholesterol HDL in serum and plasma

ORDER INFORMATION

REF	Kit size
GA4375 00	1x45 + 1x15 ml
GA4377 00	2x45 + 2x15 ml
KL4377 00	4x60 + 4x20 ml
BK4377 00	2x(40+12 ml)

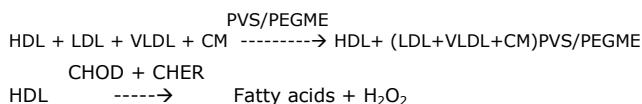
INDICATION

High-density lipoproteins (HDL) compose one of the major classes of plasma lipoproteins. They are synthesized in liver as complexes of apolipoprotein and phospholipids and are capable of picking up cholesterol and carrying it from arteries to the liver, where the cholesterol is converted to bile acids and excreted into the intestine.

An inverse relationship between HDL-cholesterol (HDL-C) levels in serum and the incidence/prevalence of coronary heart disease (CHD) has been demonstrated in a number of epidemiological studies. Measurement of HDL cholesterol is of vital importance when assessing patient's risk for CHD.

METHOD PRINCIPLE

LDL, VLDL and chylomicrons (CM) bind to PVS and PEGME present in Reagent A forming a (LDL+VLDL+CM)PVS/PEGME complex. Only HDL cholesterol reacts with the enzymes CHOD and CHER.



The quantity of quinone dye is proportional to the HDL cholesterol present in the sample.

PVS = Polyvinyl sulfonic acid
 PEGME = Polyethylene-glycol-methyl ester
 CHOD = Cholesterol oxidase
 CHER = Cholesterol esterase
 POD = Peroxidase
 AA = 4-aminoantipyrine
 TODB = N, N-Bis(4-sulfobutyl)-3-methylaniline

COMPOSITION

REAGENT A:

MES buffer, pH 6.5	50 mmol/l
TODB	2 mmol/l
PVS	2 mmol/l
PEGME	0.05 mmol/l
MgCl ₂	1 mmol/l
EDTA	1 mmol/l

REAGENT B:

MES buffer, pH 6.5	50 mmol/l
CHER	50 KU/l
CHOD	30 KU/l
POD	30 KU/l
4-AA	4 mmol/l
Detergent	0.003%

Preparation of reagents

The reagents are liquids ready to use.

Storage and stability

Store at 2-8 °C. Do not freeze the reagents! The reagents are stable up to the expiry date stated on the label, if contamination and evaporation are avoided, protected from light. The above conditions are valid if the vials are opened just only for the time to take the reagent, closed immediately with their cap and stored at the indicated conservation temperature.

ANCILLARY EQUIPMENT

- Automatic pipettes
- Photometer
- Analysis cuvettes (optical path = 1 cm)
- Temperature controlled water bath
- NaCl solution 9 g/l
- HDL/LDL Calibrator (Ref. GD0381 00)

SAMPLES

Use fresh fasting patient serum and plasma sample (EDTA, Citrate, Li-heparin).

Specimen collection / Preanalytical factors

It is recommended that specimen collection should be carried out in accordance with NCCLS Document H11-A3.

INTERNAL QUALITY CONTROL

It is recommended to use commercial control sera with known HDL cholesterol values. Check that the values obtained are within the reference range provided.

ANALYTICAL PROCEDURE

Working temperature	37 °C
Wavelength	600 nm
Optical path	1 cm
Reaction	End point

Allow the reagents to reach working temperature before using.

Pipette into disposable or well clean cuvettes :

	Blank	Calibrator	Sample
Reagent A	300 µl	300 µl	300 µl
Distilled H ₂ O	4 µl	-	-
Calibrator	-	4 µl	-
Sample	-	-	4 µl

Mix, incubate **5 minutes**. Read absorbance (A₁) of calibrator and samples against Blank. Then add:

Reagent B	100 µl	100 µl	100 µl
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Mix and incubate for **5 minutes**. Read absorbance (A₂) of calibrator and samples against Blank.

Note

- Reaction volumes can be proportionally changed.
- For values upper than 220 mg/dl dilute samples with saline solution and multiply result by the dilution factor.

CALCULATION OF RESULTS

$$\text{HDL Cholesterol, mg/dl} = \frac{(A_2 - A_1) \text{ sample}}{(A_2 - A_1) \text{ calibrator}} \times \text{mg/dl calibrator}$$

Conversion factor

$$\text{HDL Cholesterol [mg/dl]} \times 0.02586 = \text{HDL Cholesterol [mmol/l]}$$

REFERENCE VALUES

Considering the risk factor for heart disease, the expected values are the following:

High risk subjects:	< 40 mg/dl
Moderate risk subjects:	40÷59 mg/dl
No risk subjects:	≥ 60 mg/dl

Each laboratory should establish reference ranges for its own patients population.

ANALYTICAL PERFORMANCES

Precision

Within-run and between-run coefficients of variation have been calculated on replicates of three samples at different HDL cholesterol concentration. The obtained results are reported in the following tables:

Within-run				
Sample	n	Mean (mg/dl)	SD	%CV
Serum # 1	80	29.00	0.30	1.0
Serum # 2	80	53.07	0.41	0.8
Serum # 3	80	90.56	0.84	0.9

Between-run				
Sample	n	Mean (mg/dl)	SD	%CV
Serum # 1	80	29.00	0.65	2.3
Serum # 2	80	53.07	1.36	2.6
Serum # 3	80	90.56	2.02	2.2

Linearity

The assay is linear up to 220 mg/dl.

Sensitivity

Test sensitivity, in terms of limit of detection, is 1 mg/dl.

Correlation

A correlation study comparing the present method an a commercial one gave the following results:

$$y = 1.048x - 4.69 \text{ mg/dl} \quad r = 0.987$$

Interferences

Bilirubin	> 40 mg/dl
Triglycerides	> 1000 mg/dl
Ascorbic acid	> 10 mmol/l
Hemoglobin	> 1000 mg/dl

PRECAUTIONS IN USE

The reagents contain inactive components such as preservatives (Sodium azide or others), surfactants etc. The total concentration of these components is lower than the limits reported by 67/548/EEC and 88/379/EEC directives about classification, packaging and labelling of dangerous substances. However, the reagents should be handled with caution, avoiding swallowing and contact with skin, eyes and mucous membranes. The use of laboratory reagents according to good laboratory practice is recommended.

Waste Management

Please refer to local legal requirements.

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 **Manufacturer:**

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