HDL DIRECT

CLEARANCE

Intended use:

Enzymatic in vitro assay for the direct quantitative determination of HDL-cholesterol in human serum and plasma.

Summary:

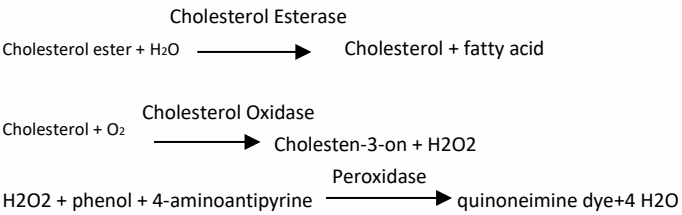
HDL (High Density Lipoproteins) are responsible for the reverse transport of cholesterol from the peripheral cells to the liver. Here, cholesterol is transformed to bile acids which are secreted into the intestine via the biliary tract. Monitoring of HDL- cholesterol in serum is of clinical importance since an inverse correlation exists between serum HDL- cholesterol concentrations and the risk of atherisclerotic disease. Elevated HDL- cholesterol concentrations are protective against coronary heart disease, while reduced HDL- cholesterol concentrations, particularly in conjunction with elevated triglycerides, increase the cardiovascular risk. A variety of methods are available to determine HDL-cholesterol, including ultracentrifugation, electrophoresis, HPLC, and precipitation-bases methods. Of these precipitation-based methods are used routinely. HDL cholesterol is first separated by precipitating apoprotein B-containing lipoproteins from serum by using a combination of a polyanion and a divalent cation, such as dextran sulfate/magnesium chloride or phosphotungstate/magnesium chloride. Such precipitation -bases method are, however, time consuming and not amenable to automated analysis. Thus, there is a great clinical need for a convenient and reliable method for measuring HDL-cholesterol in serum without any pretreatment. Several approaches for direct measurement of HDL-cholesterol in serum have been proposed, including the use of magnetically responsive particles as polyanionmetal combinations and the use of polyethylene glycol (PEG) with antiapoprotein B and anti-apoprotein CIII antibodies.

Test principle:

Enzymatic colorimetric test

- Sample and addition of R1
- Addition of R2 and start of reaction

In the first step LDL, VLDL and Chylomicrons are eliminated and transformed to non reactive compounds and specific condition for the reaction. By the second reagent only the HDL-Cholesterol is subject to color reaction



Working solution concentration:

R1:

Good's buffer, pH	7.0 100 mmol/l
Cholesterol oxidase	>0.8 KU/l
Cholesterol esterase	>1.0 KU/l
Catalase	>500 KU/l
HDCBS	0.5 mmol/l

R2:

Peroxidase	30 KU/l
4-Aminoantipyrine	4 mmol/l

Notes:

For in vitro diagnostic use.

Exercise the normal precautions required for handling all laboratory reagents

Preparation and stability:

R1: Ready for use.

R2: Ready for use.

Unopened kit components: Up to the expiry date at 2-8°C protected from light.

After first opening of R1/R2: up to 1 month at 2-8°C if contamination is avoided.

On board stability:

R1: 28 day R2: 28 days

Specimen:

Collect serum using standard sampling tubes

Li-heparin and Na-heparin- Plasma

Stability: 7 days at +2°C - +8°C
30 days at - 70°C

Fasting and non fasting samples can be used. EDTA plasma causes decreases results.

Limitations - interference:

Criterion: Recovery within ±10% of initial value.

Icterus: No significant interference up to an index I of 79 (approximate bilirubin concentration 79 mg/dl)

Hemolysis: No significant interference up to an index H of 1000 (approximate hemoglobin concentration: 1000 mg/dl).

Lipemia (Intralipid): No significant interference up to an index L of 525 (approximate triglycerides concentration: 1050 mg/dl).

There is poor correlation between turbidity and triglycerides concentration. The claim for lipemia interference is based on the Glick model, which uses Intralipid as an artificial substrate. To date, there is no model available which can mimic interference by triglycerides, as triglyceride levels in patient specimens behave unpredictably, depending on the nature of the esterified fatty acids in the samples. Patient specimens with elevated triglyceride levels are very often lipemic. Therefore customers cannot verify interference by triglycerides in patient specimens.In rare cases, elevated immunoglobulin concentrations can lead to falsely increased HDL- cholesterol results. Abnormal liver function does affect lipid metabolism; consequently, HDL and LDL results are of limited diagnostic value. In some patients with abnormal liver function, the HDL-D result is significantly negatively biased versus the DCM (designated comparison method) result.

Testing procedure:

Applications for automated systems are available on request.

Materials provided

- Working solutions as described above

Additional materials required

- Calibrators and controls as indicated below
- 0.9% NaCl

Manual Testing	
Wavelength:	Hg 570 nm (side wavelength 700 nm)
Reaction temperature:	+37°C
Cuvette:	1 cm light path
Zero adjustment	Sample blank
	Sample/Calib./Stand.
Sample/ Calib./Stand.	3 µl
R1	300 µl
Mix well and incubate at 37°C for 5 minutes and read blank	
R2	100 µl
Incubate at 37°C for 5 minutes. Read the sample absorbance. Calculate A = Asample-Ablank	

Measuring/reportable range:

7,75 - 100 mg/dl

Determine samples having higher concentrations via the rerun function. On instruments without rerun function, manually dilute the samples with 0.9% NaCl
(e.g. 1 + 1). Multiply the result by the appropriate dilution factor (e.g. factor 2).

Expected values:

Adults: 30 – 60 mg/dL

Expected values may vary with age, sex, diet, and geographical location. Each laboratory should determine its own expected values as dictated by good laboratory practice.

1. < 40 mg/dL as indicative of a major risk factor for Coronary Heart Disease.
2. > 60 mg/dL as a negative risk factor for Coronary Heart Disease.

Analytical sensitivity (lower detection limit)

Detection limit: 7,75 mg/dl

The lower detection limit represents the lowest measurable HDL-cholesterol concentration that can be distinguished from zero.

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Imprecision:

Reproducibility was determined using controls in an internal protocol.
The following results were obtained:

Within run			
Sample	Mean (mg/dl)	SD (mg/dl)	% CV
Control Serum 1	25.57	0.706	2.76
Control Serum 2	38.32	0.703	1.83
Control Serum 3	48.40	0.506	1.05

Method comparison:

A comparison of the BIOANALYTIC HDL-D (y) with a commercial obtainable assay (x) gave the following result (mg/dl):
y = 0.917x + 4.313 ; r = 0.975

Quality Control:

Control Serum:

BIOCON N	5 x 5 ml	#B10814
BIOCON P	5 x 5 ml	#B10817

The control intervals and limits must be adapted to the individual laboratory and country-specific requirements. Values obtained should fall within established limits. Each laboratory should establish corrective measures to be taken if values fall outside the limits.

Calibration:

S1: 0.9% NaCl

S2: BIOCAL H	5 x 3 ml	#B11895

Calibration stability:

It is suggested to use Calibrator products produced by Bioanalytic. It is suggested to use supplementary calibrator (pure water) to conduct 2-point calibration. The calibration curve is formed automatically. When lot number is changed or QC is invalid, calibration shall be conducted again. Recalibrate the assay every 30 days under ideal conditions,
or when the following occur:
Change in reagent lot or significant shift in control values;
Major preventative maintenance was performed on the analyser or a critical part was replaced(Halogen Lamp).

Order information (Cat No.) :

CC420	AB421	B24180	B28180	B32180	B35181	B80183
CC421	BHDL400	B24181	B28181	B32181	B36180	
OL420	BHDL200	B25180	B28182	B33180	B36181	
OL421	BHDL100	B25181	B30180	B33181	B37180	
KL420	B21180	B25182	B30181	B33182	B37181	
KL421	B21181	B27180	B30182	B34180	B80180	
CR420-421	B21182	B27181	B31180	B34181	B80181	
AB420	B22180	B27182	B31181	B35180	B80182	

Literature:

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Manufacturer

Diaclinica Diagnostik Kimya.San.Tic.Ltd.Şti
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Web :www.diaclinica.com

SYMBOLS

IVD	for in vitro diagnostic use only
LOT	lot of manufacturing
REF	code number
	storage at temperature interval
	expiration date (year/month)
	warning, read enclosed documents
	Read the directions

ISO 9001:2015
ISO 13485:2016