CORMAY HDL DIRECT

II GENERATION

DIAGNOSTIC KIT FOR DETERMINATION OF HDL-CHOLESTEROL CONCENTRATION (DIRECT METHOD)

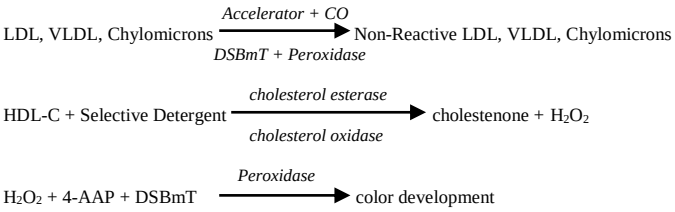
Kit name	**Cat. No**
CORMAY HDL DIRECT 500	2-184
CORMAY HDL DIRECT "bulk"	2-226

INTRODUCTION

Plasma lipoproteins are spherical particles containing varying amounts of cholesterol, triglycerides, phospholipids and proteins. The relative protein and lipid determine the density of these lipoproteins and provide the basis on which to begin their classification. The classes are: chylomicron, very-low-density lipoprotein (VLDL), low-density-lipoprotein (LDL) and high-density lipoprotein (HDL). The principle role of HDL in lipid metabolism is the uptake and transport of cholesterol from peripheral tissues to the liver. Low HDL cholesterol (HDL-C) levels are strongly associated with an increased risk of coronary artery disease. The HDL-C determination is used to identify high-risk patients.

METHOD PRINCIPLE

The assay is a homogeneous method for directly measuring HDL-cholesterol concentration in serum or plasma, without any off-line pretreatment or centrifugation steps.
Accelerator selective detergent methodology.
During the first phase, LDL, VLDL particles and Chylomicrons generate free non-HDL cholesterol, which through an enzymatic reaction, produce hydrogen peroxide. The generated peroxide is consumed by a peroxidase reaction with DSBmT yielding a colourless product.
During the second phase, specific detergent solubilises HDL-Cholesterol. In conjuction with cholesterol oxidase (CO) and cholesterol esterase (CE) action, peroxidase and 4-AAP develop a coloured reaction which is proportional to HDL-Cholesterol concentration.



REAGENTS

Package

	CORMAY HDL DIRECT 500	**CORMAY HDL DIRECT "bulk"**
1-Reagent	3 x 300 ml	--*
2-Reagent	1 x 300 ml	--*

* reagent volume is printed on the label.

The reagents are stable up to the kit expiry date printed on the package when stored at 2-8°C. On board stability of the reagents depends on type of analyser used for analysis. Protect from light and avoid contamination!

Concentrations in the test

1-Reagent

Buffer	
Cholesterol oxidase (*E.coli*)	< 1000 U/l
Peroxidase (horseradish)	< 1300 ppg U/l
N,N-bis(sulfobutyl)-toluidine, disodium (DSBmT)	< 1 mM
Accelerator	< 1 mM
Preservative	< 0.06 %
Ascorbic acid oxidase (*Curcubita sp.)*	< 3000 U/l

2-Reagent

Buffer	
Cholesterol esterase (*Pseudomonas sp*.)	< 1500 U/l
4–aminoantipyrine (4-AAP)	< 1 mM
Detergent	< 2 %
Preservative	< 0.06 %

Warnings and notes

- Product for in vitro diagnostic use only.

SPECIMEN

Serum, heparinized or EDTA plasma.
Anticoagulants containing citrate should not be used.
Blood should be collected only if the patient has been fasting for 12 -14 hours.
Serum: Collect whole blood by venepuncture and allow to clot. Centrifuge and remove the serum as soon as possible after collection (within 3 hours).
Plasma: Specimens may be collected in EDTA or lithium or sodium heparin. Centrifuge and remove the plasma as soon as possible after collection (within 3 hours).
Serum and plasma should not remain at 15-30°C longer than 14 hours. If assays are not completed within 14 hours, serum or plasma should be stored at 2 - 8°C for up to 1 week. If specimens need to be stored for more than 1 week, they may be preserved at less than -20°C for up to 3 months. Samples may be frozen once.
Nevertheless it is recommended to perform the assay with freshly collected samples!

ADDITIONAL EQUIPMENT

- automated analyser or photometer able to read at 630 nm;
- thermostat at 37ºC;
- general laboratory equipment;

PROCEDURE

These reagents may be used for manual assay and in several automatic analysers. Applications for them are available on request. The reagents are ready to use.

Manual procedure

wavelength	630 nm
temperature	37°C
cuvette	1 cm
reaction type	Endpoint

Pipette into the cuvette:

	Test (T)	standard (S)
1-Reagent	1200 µl	1200 µl

Bring up to the temperature of determination (37 °C). Then add:

calibrator	-	10 µl
sample	10 µl	-

Mix well, incubate for 5 min at 37°C. Then add:

2-Reagent	400 µl	400 µl

Mix well and incubate at the temperature of determination. After 7 minutes read the absorbance of test A(T) and standard A(S) against air or water.

Calculation

$$\text{HDL Direct concentration} = \frac{A\,(T)}{A\,(S)} \times \text{calibrator concentration}$$

REFERENCE VALUES[4]

serum / plasma	40 – 60 mg/dl 1.04 – 1.55 mmol/l

As HDL cholesterol is affected by a number of factors such as smoking, exercise, hormones, age and sex, each laboratory should establish its own reference ranges for local population.

QUALITY CONTROL

For internal quality control it is recommended to use CORMAY LIPID CONTROL 1 (Cat. No 5-179) and CORMAY LIPID CONTROL 2 (Cat. No 5-180) or CORMAY SERUM HN (Cat. No 5-172) and CORMAY SERUM HP (Cat. No 5-173) with each batch of samples.
For the calibration of automatic analysers the CORMAY HDL/LDL CALIBRATOR (Cat. No 5-178) is recommended.
Calibration stability depends on type of analyser used for analysis.
The calibration curve should be prepared with change of reagent lot number or as required e.g. quality control findings outside the specified range.

PERFORMANCE CHARACTERISTICS

These metrological characteristics have been obtained using Epoll 20, Multi+ for manual assay and automatic analyser Biolis 24i Premium. Results may vary if a different instrument is used.

- **Sensitivity (Epoll 20):** 11.5 U/l (0.298 µkat/l).
  Sensitivity (Biolis 24i Premium): 1.1 mg/dl (0.028 mmol/l).

- **Linearity (Epoll 20):** up to 282 mg/dl (7.30 mmol/l).
  Linearity (Biolis 24i Premium):up to 200 mg/dl (5.18 mmol/l).
  For higher concentration of HDL cholesterol dilute the sample with physiological saline before assaying. Multiply the result obtained from the manual dilution by the appropriate dilution factor.

- **Specificity / Interferences**
  Bilirubin conjugated up to 60 mg/dl, bilirubin total up to 60 mg/dl, haemoglobin up to 1 g/dl, ascorbic acid up to 100 mg/dl, Intralipid up to 1800 mg/dl, triglycerides up to 2000 mg/dl and gamma-globulins up to 5000 mg/dl do not interfere with the test.

- **Precision (Multi+)**

Repeatability (run to run) n = 10	Mean [mg/dl]	SD [mg/dl]	CV [%]
level 1	39.51	1.24	3.13
level 2	69.63	0.75	1.08

- **Precision (Biolis 24i Premium)**

Repeatability (run to run) n = 10	Mean [mg/dl]	SD [mg/dl]	CV [%]
level 1	43.30	0.61	1.42
level 2	58.20	0.88	1.51

Reproducibility (day to day) n = 10	Mean [mg/dl]	SD [mg/dl]	CV [%]
level 1	43.91	1.68	3.84
level 2	58.02	1.06	1.83

- **Method comparison**

A comparison between HDL cholesterol values determined at Epoll 20 (y) and at Biolis 50i (x) using 24 samples gave following results:
y = 1.0578 x - 4.4324 mg/dl;
R= 0.958 (R - correlation coefficient)

A comparison between HDL cholesterol values determined at Biolis 24i Premium (y) and at ADVIA 1650 (x) using 58 samples gave following results:
y = 0.8436 x + 3.2579 mg/dl;
R= 0.984 (R - correlation coefficient)

WASTE MANAGEMENT

Please refer to local legal requirements.

LITERATURE

1. Gotto, A.M. Lipoprotein metabolism and the etiology of hyperlipidemia. Hospital Practice 1988; 23 Suppl:1 4-13.
2. Badimon, J. J., Badimon, L., Fuester V. Regression of Atherosclerotic Lesions by High Density Lipoprotein Plasma Fraction in the Cholesterol-Fed Rabbit. J Clin Invest 1990; 85:1234-41.
3. Warnick, G. Russell, Wood, Peter D. National Cholesterol Education Program Recommendations for Measurement of High-Density Lipoprotein Cholesterol: Executive Summary. Clin Chem 1995; 41(10):1427-1433.
4. Alan H.B. Wu: Tietz Clinical Guide to Laboratory Tests, 4th ed. WB Saunders, 564 (2006).
5. Camps, J, Altered Composition of Lipoproteins in Liver Cirrhosis Compromises Three Homogeneous Methods for HDL-Cholesterol, Clinical Chemistry, 1999; 45:685-688.

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MANUFACTURER

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