CORMAY LDL DIRECT

II GENRATION

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

Kit name	Cat. No
CORMAY LDL DIRECT 500	2-194
CORMAY LDL DIRECT "bulk"	2-195

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).
LDL are synthesised in the liver by the action of various lipolytic enzymes on triglyceride rich VLDL. LDL-cholesterol concentration is considered to be the most important clinical predictor, of all single parameters, with respect to coronary atherosclerosis.
Accurate measurement of LDL-cholesterol is of vital importance in therapies which focus on lipid reduction to prevent atherosclerosis or reduce its progress and to avoid plaque rupture.

METHOD PRINCIPLE

The assay is a homogenous method for directly measuring LDL-cholesterol concentrations in serum or plasma, without the need for any off-line pretreatment or centrifugation steps.
Liquid selective detergent method.
The method is in a two reagents format and depends on the properties of a unique detergent. This detergent (Reagent 1) solubilizes only the non LDL particles (HDL, VLDL, CM). The cholesterol released is consumed by cholesterol esterase and cholesterol oxidase in a non color forming reaction. A second detergent (Reagent 2) solubilizes the remaining LDL particles and chromogenic coupler allows for color formulation. The enzyme reaction with LDL-cholesterol in the presence of the coupler produces color that is proportional to the amount of LDL cholesterol present in the sample.

REAGENTS

Package

	CORMAY LDL DIRECT 500	CORMAY LDL DIRECT "bulk"
1-Reagent	1 x 3000 ml	--*
2-Reagent	2 x 500 ml	--*

* reagent volume is printed on the label.

The reagent is stable up to the kit expiry date printed on the package when stored at 2-8°C. The reagents are stable for 12 weeks on board the analyser at 2-10°C. Do not freeze reagents. Protect from light and avoid contamination!

Concentrations in the test

1-Reagent

Buffer	
Detergent 1	< 1.0 %
Cholesterol esterase (*Pseudomonas sp.)*	< 1500 U/l
Cholesterol oxidase (*Cellulomonas sp.)*	< 1500 U/l
Peroxidase (horseradish)	< 1300 ppg U/l
4-aminoantipyrine	< 0.1 %
Ascorbic Acid Oxidase (*Curcubita sp.*)	< 3000 U/l
Preservative	

2-Reagent

Buffer	
Detergent 2	< 1.0 %
N,N-bis(sulfobutyl)-toluidine, disodium (DSBmT)	< 1.0 mM
Preservative	

2-Reagent

GOOD's buffer	10 mmol/l
N, N-Bis-(4-sulfobutyl)-3-methylaniline, disodium salt (TODB)	2 mmol/l
preservative	0.5 g/l
detergent	1 %

Warnings and notes

- Product for in vitro diagnostic use only.

SPECIMEN

Serum, sodium heparinized or EDTA plasma.
Anticoagulants containing citrate should not be used.
Serum: Collect whole blood by venipuncture 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 sodium heparin. Centrifuge and remove the plasma as soon as possible after collection (within 3 hours).
If not analysed promptly, specimens may be stored at 2-8°C for up to 5 days. If specimens need to be stored for more than 5 days, they may be preserved at -80°C. 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 both 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

Pipette into the cuvettes:

	test (T)	standard (S)
1-Reagent	900 µl	900 µl
Bring up to the temperature of determination (37 °C). Then add		
calibrator	-	9 µl
sample	9 µl	-
Mix well and after 5 min. of incubation at 37ºC add:		
2-Reagent	300 µl	300 µl

Mix well and after exactly 5 min. of incubation read the absorbance of standard A(S) and test A(T) against air or water.

Calculation

$$\text{LDL cholesterol concentration} = \frac{A(T)}{A(S)} \times \text{calibrator concentration}$$

REFERENCE VALUES [7]

NCEP* Classification:	Adults	
Optional	< 100 mg/dl	< 2.59 mmol/l
Near optional	< 130 mg/dl	< 3.37 mmol/l
Bordeline high	130-159 mg/dl	3.37-4.12 mmol/l
High	160-189 mg/dl	4.14-4.90 mmol/l
Very high	≥ 190 mg/dl	≥ 4.92 mmol/l

* National Cholesterol Education Program

As LDL 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 the CORMAY HDL/LDL CALIBRATOR (Cat. No 5-178) is recommended.
The calibration curve should be prepared every 12, 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 Multi+ for manual assay and automatic analyser Biolis 24i Premium. Results may vary if a different instrument is used.

- **Sensitivity (Multi+):** 5.5 U/l (0.11 µkat/l).
  Sensitivity (Biolis 24i Premium): 4.2 mg/dl (0.028 mmol/l).

- **Linearity (Multi+):** up to 316 mg/dl (8.18 mmol/l).
  Linearity (Biolis 24i Premium):up to 700 mg/dl (18.13 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**
  Triglycerides up to 1293 mg/dl, bilirubin conjugated up to 20 mg/dl, bilirubin total up to 20 mg/dl, haemoglobin up to 0.5 g/dl, ascorbic acid up to 500 mg/l 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	62.72	1.22	1.94
level 2	158.77	0.69	0.44

Precision (Biolis 24i Premium)

Repeatability (run to run) n = 10	Mean [mg/dl]	SD [mg/dl]	CV [%]
level 1	135.60	4.35	3.21
level 2	187.07	5.19	2.77

Reproducibility (day to day) n = 10	Mean [mg/dl]	SD [mg/dl]	CV [%]
level 1	135.06	3.67	2.71
level 2	187.24	5.62	3.00

- **Method comparison**

A comparison between HDL cholesterol values determined at Multi+ (y) and at Hitachi 912 (x) using 48 samples gave following results:
y = 0.8968 x + 11.543 mg/dl;
R= 0.982 (R - correlation coefficient)

A comparison between HDL cholesterol values determined at Biolis 24i Premium (y) and at Hitachi 912 (x) using 29 samples gave following results:
y = 1.0365 x + 3.7224 mg/dl;
R= 0.996 (R - correlation coefficient)

WASTE MANAGEMENT

Please refer to local legal requirements.

LITERATURE

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