Cat. No 7-480

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

A-400 LDL DIRECT

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 synthesized 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

1-Reagent	2 x 29 ml
2-Reagent	2 x 10,5 ml

The reagents are 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.

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	

Warnings and notes

- Product for in vitro diagnostic use only.
- Do not freeze reagents. Protect from light and contamination!
- The reagents must be used only for the intended purpose, by suitably qualified laboratory personnel, under appropriate laboratory conditions.

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.

PROCEDURE

These reagents may be used in automatic analysers BS-400 and BS-480.
1-Reagent and 2-Reagent are ready to use.
For reagent blank deionized water is recommended.

Actions required:

BS-400

When performing assays at analyser BS-400, there is a probability of **cross-contamination** affecting the tests results: LDL DIRECT II GEN – LIPASE II GEN, LDL DIRECT II GEN – URINE PROTEINS II GEN, LDL DIRECT II GEN – TGmono, LDL DIRECT II GEN – LIPASE. To avoid this effect follow the recommendations contained in the advisory note **"Carry-over – Preventive Actions "**.

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 of automatic analysers the CORMAY HDL/LDL CALIBRATOR (Cat. No 5-178) is recommended. Deionized water should be used as a calibrator 0.
The calibration curve should be prepared every 12 weeks, 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 the automatic analysers BS-400 and BS-480. Results may vary if a different instrument or a manual procedure is used.

- **Sensitivity:**

4 mg/dl (0.10 mmol/l) – BS-400
2 mg/dl (0.05 mmol/l) – BS-480

- **Linearity:**

up to 700 mg/dl (18.13 mmol/l) – BS-400
up to 940 mg/dl (24.35 mmol/l) – BS-480
Patient samples with LDL cholesterol levels exceeding 700 mg/dl should be diluted 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**

Repeatability (run to run)		Mean [mg/dl]	SD [mg/dl]	CV [%]
BS-400 (n = 10)	level 1	131.05	4.01	3.06
	level 2	185.93	4.29	2.31
BS-480 (n = 10)	level 1	127.46	0.97	0.76
	level 2	172.87	0.94	0.54
Reproducibility (day to day)		Mean [mg/dl]	SD [mg/dl]	CV [%]
BS-400 (n = 10)	level 1	131.68	3.45	2.62
	level 2	186.85	5.33	2.85
BS-480 (n = 10)	level 1	48.66	0.71	1.47
	level 2	132.35	2.57	1.94

- **Method comparison**

A comparison between CORMAY reagent (y) and another commercially available assay (x) using 29 samples at analysers Hitachi 912 and BS-400 gave following results:
y = 0.9768 x + 3.2127 mg/dl;
R= 0.996 (R - correlation coefficient)

A comparison between LDL values determined at BS-480 (y) and at BS-800 (x) using 39 samples gave following results:
y = 1.0176 x – 0.3952 U/l;
R = 1.000 (R – correlation coefficient)

WASTE MANAGEMENT
Please refer to local legal requirements.

LITERATURE

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Date of issue: 12. 2017.

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12/17/12/17