Cat. No 4-569

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

# HC – 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).

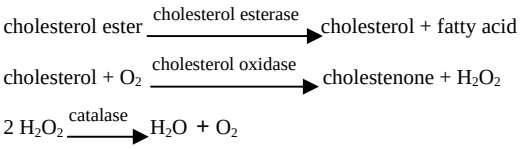
LDL are synthesized in the liver by the action of various lipolytic enzymes on triglyceride rich VLDL. LDL-cholesterol concentration is considered to be the most important clinical predictor, of all single parameters, with respect to coronary atherosclerosis.

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.

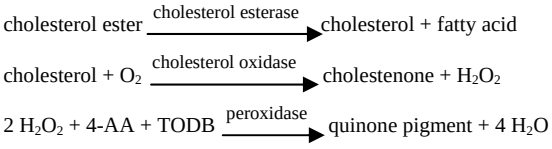
## METHOD PRINCIPLE

The assay consists of 2 distinct reaction steps:

1. Elimination of chylomicron, VLDL and HDL by cholesterol esterase, cholesterol oxidase and subsequently catalase.




2. Specific measurement of LDL-Cholesterol after release of LDL- Cholesterol by detergents in 2-Reagent.




The colour intensity measured spectrophotometrically is proportional to the LDL cholesterol concentration.

## REAGENTS

**Package**

| | |
|---|---|
| 1-Reagent | 2 x 57 ml |
| 2-Reagent | 2 x 19 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. Do not freeze reagents. Protect from light and avoid contamination!

**Concentrations in the test**

| | |
|---|---|
| **1-Reagent** | |
| GOOD's buffer | 10 mmol/l |
| cholesterol esterase | 5 KU |
| cholesterol oxidase | 5 KU |
| 4-aminoantipyrine (4-AA) | 0.5 g/l |
| peroxidase | 20 KU |
| MgCl2 | 2 mmol/l |
| detergent | 0.5 g/l |
| preservative | 0.5 g/l |
| **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.
- Do not use after expiry date.
- Do not interchange caps.
- Reagents should be mixed before use by gentle inverting the bottle several times.

## SPECIMEN

Serum, heparinized or EDTA plasma.

Do not use citrate, oxalate and fluoride as anticoagulants.

Blood should be collected only if the patient has been fasting for 12 – 16 hours.

Serum and plasma can be stored up to 7 days at 4°C. Sample are stable for 1 month when stored at -20°C and for 1 year when stored at -70°C. Avoid repeated freezing and thawing.

Nevertheless it is recommended to perform the assay with freshly collected samples!

## PROCEDURE

The reagents are ready to use.

These reagents may be used in automatic analyser Hitachi 911/912.

Application should be entered using handheld barcode scanner and attached barcodes sheet, according to procedure described below:

1. Delete previous version of application and calibrators assigned to it and restart the analyser.
2. Enter codes of calibrators according to the attached list.
3. Enter barcoded application and assign proper values to calibrators.
4. To activate entered application go to the tab UTILITY | APPLICATION | RANGE and change value of field DATA MODE from INACTIVE to ON BOARD. Confirm the change using UPDATE button.
5. Put reagents on board the analyser – they will be assigned to relevant tests automatically. Perform also measurement of level of reagents inside the bottles.
6. After calibration analyser is ready to use.

## REFERENCE VALUES [7]

| | |
|---|---|
| serum / plasma | < 100 mg/dl<br>< 2.59 mmol/l |

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. **Calibrator and 0.9% NaCl** should be used for calibration.

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 analyser Hitachi 912. Results may vary if a different instrument or manual procedure is used.

- **Sensitivity:** 2 mg/dl (0.052 mmol/l).

- **Linearity:** up to 430 mg/dl (11.14 mmol/l).
  For higher concentration dilute the sample with 0.9% NaCl and repeat the assay. Multiply the result by dilution factor.

- **Specificity / Interferences**
  Haemoglobin up to 0.5 g/dl, triglycerides up to 1000 mg/dl, ascorbate up to 50 mg/dl and bilirubin up to 20 mg/dl do not interfere with the test.

- **Precision**

| Repeatability (run to run) n = 20 | Mean [mg/dl] | SD [mg/dl] | CV [%] |
|---|---|---|---|
| level 1 | 58.00 | 0.34 | 0.59 |
| level 2 | 136.90 | 1.57 | 1.14 |

| Reproducibility (day to day) n = 80 | Mean [mg/dl] | SD [mg/dl] | CV [%] |
|---|---|---|---|
| level 1 | 61.58 | 2.90 | 4.71 |
| level 2 | 142.33 | 4.92 | 3.50 |

- **Method comparison**

A comparison between LDL cholesterol values determined at Hitachi 912 (y) and at COBAS INTEGRA 400 PLUS (x) using 81 samples gave following results:
$y = 0.9902 x + 6.125$ mg/dl;
$R = 0.9813$ (R – correlation coefficient)

**WASTE MANAGEMENT**
Please refer to local legal requirements.

**LITERATURE**

1. Natio H.K., et al, Clin Chem, 41: 132-133, 1995.
2. Weiland H. and Seidel D., J Lip Res, 24: 904 – 909, 1983.
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6. Crouse J.R. et al.: J. Lipid Res., 26; 566-574 (1985).
7. Alan H.B. Wu: Tietz Clinical Guide to Laboratory Tests, 4th ed. WB Saunders, 684 (2006).


**Date of issue:** 04. 2012.


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