CORMAY HDL

DIAGNOSTIC KIT FOR DETERMINATION OF HDL-CHOLESTEROL CONCENTRATION

Kit name	Cat. No
CORMAY HDL 500	2-055
CORMAY HDL "bulk"	2-054

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) levels are strongly associated with an increased risk of coronary artery disease.

METHOD PRINCIPLE

Very low (VLDL) and low density (LDL) lipoproteins contained in the sample are precipitated by the addition of phosphotungstic acid in the presence of magnesium ions. The supernatant obtained after centrifugation contains high density lipoproteins (HDL) which can be determined enzymatically.

REAGENTS

Package

	CORMAY HDL 500	CORMAY HDL "bulk"
1-PRECIPITANT	4 x 500 ml	--*

*reagent volume is printed on the label.

Reagent preparation and stability

The reagents are ready to use.

The reagents when stored at 2-8°C are stable up to expiry date printed on the package.

Concentrations in the test

phosphotungstic acid	32.0 g/l
magnesium chloride	61.0 g/l
stabilizers	3.2 g/l

Warnings and notes

- Product for in vitro diagnostic use only.
- For determination HDL-cholesterol concentration in supernatant the Liquick Cor-CHOL mini/30/60/120 (cat. no 2-212, 2-211, 2-204, 2-205) is recommended.
- Standard HDL – 1.3 mmol/l (50 mg/dl) is available on separate request.
- The standards contain 0.09% sodium azide as a preservative. Avoid contact with skin and mucous membranes.
- The centripetal acceleration value (4000 x g) should be recalculated into rpm (revolutions per minute). The recalculation factor depends on the size of used centrifuging rotor.
- Prepare the working reagent according to the kit inserts-Liquick Cor-CHOL.
- 1-Precipitant meeting the criteria for classification in accordance with Regulation (EC) No 1272/2008.

Ingredients:

1-Precipitant contains phosphotungstic acid hydrate.

Danger

H318 Causes serious eye damage.

H315 Causes skin irritation.

P280 Wear protective gloves/protective clothing/eye protection/face protection.

P305 +P351 +P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P310 Immediately call a POISON CENTER or doctor/physician.

P302+ P352 IF ON SKIN: Wash with plenty of soap and water.

ADDITIONAL EQUIPMENT

- centrifuge;
- diagnostic kit for determination of cholesterol concentration (eg. Liquick Cor-CHOL mini/30/60/120);
- automatic analyzer or photometer able to read at 500 nm (Hg 546 nm);
- thermostat at 37ºC;
- general laboratory equipment;

SPECIMEN

Serum, EDTA or heparinized plasma free from hemolysis.

Blood should be collected only if the patient has been fasting for 12-14 hours.

The sample should be removed from clot within 2 hours and stored at 4°C until analysis.

PROCEDURE

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

Sample preparation:

Pipette into centrifuge tubes:

Sample	500 µl
1-PRECIPITANT	50 µl

Mix well, allow to stand for 10 min. at room temperature and centrifuge for 10 min. at least 4000 x g. Attention! The centripetal acceleration value (4000 x g) should be recalculated into rpm (revolutions per minute). The recalculation factor depends on the size of used centrifuging rotor.

After centrifugation separate the clear supernatant from the precipitate and during 1 hour determine the cholesterol concentration using kit for the enzymatic total cholesterol determination (Liquick Cor-CHOL) and application for CORMAY HDL.

If the supernatant is not clear (high triglycerides level), dilute the sample with an equal volume of 0.9% NaCl solution and repeat the assay. Multiply the result by 2.

Manual procedure

wavelength	500 nm (Hg 546 nm)
temperature	37°C
cuvette	1 cm

Pipette into the cuvettes:

	reagent blank (RB)	test (T)	standard (S)
working reagent	1000 µl	1000 µl	1000 µl

Bring up to the temperature of determination. Then add:

distilled water	50 µl	-	-
supernatant	-	50 µl	-
standard	-	-	50 µl

Mix well, incubate for 5 min. at 37ºC. Read the absorbance of the test A(T) and standard A(S) against reagent blank (RB). The colour intensity is stable within 30 minutes.

Calculation

$$\text{HDL concentration} = \frac{A(T)}{A(S)} \times 1.1 \times \text{standard concentration}$$

REFERENCE VALUES

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) with each batch of samples.

PERFORMANCE CHARACTERISTICS

These metrological characteristics have been obtained using Liquick Cor-CHOL kit and automatic analyser Cobas Mira. Results may vary if a different instrument, kit or a manual procedure is used.

- **Sensitivity / Limit of Quantitation:** 4 mg/dl (0.10 mmol/l).

- **Specificity / Interferences**
  Haemoglobin up to 50 mg/dl, ascorbate up to 3 mg/dl, triglycerides up to 1200 mg/dl and bilirubin up to 4 mg/dl do not interfere with the test.

- **Precision**

Repeatability (run to run) n = 20	Mean [mg/dl]	SD [mg/dl]	CV [%]
level 1	37.07	0.45	1.20
level 2	57.93	0.88	1.53

Reproducibility (day to day) n = 20	Mean [mg/dl]	SD [mg/dl]	CV [%]
level 1	37.7	0.35	0.93
level 2	58.1	0.51	0.88

- **Method comparison**

A comparison between CORMAY reagent (y) and commercially available assay (x) using 17 samples gave following results:
y = 0.940 x – 8.162 mg/dl;
R= 0.970 (R - correlation coefficient)

TRACEABILITY

HDL STANDARD 50 is traceable to the SRM 1951B reference material.

WASTE MANAGEMENT

Please refer to local legal requirements.

LITERATURE

1. Lopez –Virella M.F et al.: Clin. Chem. 23, 882 (1977).
2. Fruchart J.C: Rev. des Labolatories 7, 103 (1982).
3. Wernick G.R. , Nguyem T., Albers A.A.: Clin. Chem. 31, 217 (1985).
4. Gordon T. et al.: Am. J. Med., 62; 707 (1977).
5. Kaplan L.A., Pesce A.J.: Clinical Chemistry – Theory, analysis and correlation. Third edition, Mosby, 1996, 674-676.
6. Wiliams P., Robinson D., Baily A.: Lancet, 1/72 (1979).
7. Goto A.M.: Hospital Practice, 23; Suppl., 1, 4 (1988).
8. Crouse J.R. et al.: J. Lipid Res., 26; 566 (1985).
9. Badmion J.J., Badmion L., Fuester V.: Journal of Clinical Investigation, 85:1234-41 (1990).
10. Castelli W.P. et all.: Circulation, 55; 767 (1977).
11. Barr D.P., Russ E.M., Eder H.A.: Am. J. Med., 11; 480 (1951).
12. Kannel W.B., Castelli W.P., Gordon T.: Ann. Intern. Med., 90:85 (1979).

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