Direct HDL
(Direct clearance method)

CHEM Count

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.

METHOD PRINCIPLE

The assay consists of 2 distinct reaction steps:

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

$$\text{cholesterol ester} \xrightarrow{\text{cholesterol esterase}} \text{cholesterol} + \text{fatty acid}$$

$$\text{cholesterol} + O_2 \xrightarrow{\text{cholesterol oxidase}} \text{cholestenone} + H_2O_2$$

$$2\,H_2O_2 \xrightarrow{\text{Catalase}} H_2O + O_2$$

2. Specific measurement of HDL-Cholesterol after release of HDL Cholesterol by detergents in R2 Reagent. In the second reaction catalase is inhibited by sodium azide in R2-Reagent.

$$\text{cholesterol ester} \xrightarrow{\text{cholesterol esterase}} \text{cholesterol} + \text{fatty acid}$$

$$\text{cholesterol} + O_2 \xrightarrow{\text{cholesterol oxidase}} \text{cholestenone} + H_2O_2$$

$$2\,H_2O_2 + \text{4-AA} + \text{HDAOS} \xrightarrow{\text{peroxidase}} \text{quinone pigment} + 4\,H_2O$$

The colour intensity is proportional to the HDL-cholesterol concentration when measured at 600 nm.

KIT CONTENTS

Reagent Name	Pack Size	Pack Size
R1 HDL Reagent	1 x 30 ml	2 x 30 ml
R2 HDL Reagent	1 x 10 ml	2 x 10 ml
R3 - Calibrator	0.5 ml	0.5 ml

R3-Calibrator will be provided, the concentration will be printed on the label.

WORKING REAGENT PREPARATION AND STABILITY

The reagents R1 & R2 are stable up to the kit expiry date printed on the package when stored at 2-8°C. Protect from light.

Calibrator: Reconstitute with required distilled water mentioned on the bottle label. Let it stand for 30 minutes at room temperature. Dissolve the content of the vial by swirling gently to avoid the formation of foam. The reconstituted calibrator is stable only for 7 days at 2-8°C.

CONCENTRATIONS IN THE TEST

Cholesterol Esterase	≥1000 U/L
Cholesterol Oxidase	≥ 800 U/L
Catalase	≥ 900 U/L
TOOS	
4 - aminoantipyrine	≥4000 U/L
peroxidase	≥3.5 KU/L
sodium azide	0.05 %
surfactants	1.4 %

WARNINGS AND NOTES

- Product for in vitro diagnostic use only.
- The reagents are ready to use.
- Do not pipette by mouth.
- The reagents contain 0.05% sodium azide as a preservative.
- Avoid contact with skin and mucous membranes.

SPECIMEN

Serum, heparinized or EDTA plasma.

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

Serum and plasma can be stored up to 6 days at 2 - 8°C. Sample are stable for 1 year when stored at for -70°C. Samples may be frozen once. If any samples show precipitates, centrifuge before using .Nevertheless it is recommended to perform the assay with freshly collected samples.

PROCEDURE

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

Wavelength	578 nm
Temperature	37°C
Cuvette	1 cm

Pipette into the cuvette:

Reagent	Blank (B)	Calibrator (C)	Test (T)
R1 HDL Reagent	450 µl	450 µl	450 µl
Distilled Water	5 µl	-	-
R3 - Calibrator	-	5 µl	-
Sample	-	-	5 µl
Mix well and incubate for 5 mins at 37° C, than add			
R2 HDL Reagent	150 µl	150 µl	150 µl

Mix well & incubate for 5 min. at 37°C. Measure the absorbance of calibrator & sample against reagent blank.

CALCULATION

$$\text{Concentration in mg/dl} = \frac{\text{Abs.Test}}{\text{Abs.Calibrator}} \times \text{Calibrator Concentration}$$

REFERENCE VALUE

Male	35 to 80 mg / dl
Female	42 to 88 mg / dl

It is recommended for each laboratory to establish its own reference ranges for local population.

QUALITY CONTROL

To ensure adequate quality control, each run should include assayed normal and abnormal controls. If commercial controls are not available it is recommended that known value samples be aliquoted, frozen and used as controls.

PERFORMANCE CHARACTERISTICS

Linearity: The procedure is linear upto 180 mg/dl. Patient samples with HDL cholesterol levels exceeding 180 mg/dl should be diluted with physiological saline before assaying. Multiply the result obtained from the manual dilution by the appropriate dilution factor.

Sensitivity / Limit of Quantitation: 0.55 mg/dl

Specificity / Interferences

Bilirubin up to 20 mg/dl, , haemoglobin up to 20 g/dl, ascorbate up to 62 mg/l and triglycerides up to 500 mg/dl do not interfere with the test.

WASTE MANAGEMENT

Please refer to local legal requirements.

LITERATURE

1. National Institutes of Health Consensus Development Conference Statement: Triglyceride, High Density Lipoprotein and Coronary Heart Disease. Washington D.C. Feb 26-28, 1992.
2. Izawa S., Okada M., Matusui H., and Horita Y. J. Medicine and Pharmaceutical Sci., 1385-1388, 37(1997).
3. Shih WJ, Bachorik PS, Haga JA, Myers GL, Stein EA; Clinical Chemistry, 2000; 46:3:351-364.
4. Third Report of the National Cholesterol Education Programme (NCEP) Expert Panel on Detection, Evaluation and treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) JAMA Publication, Vol 285, No. 19, P2486-2497; 2001.
5. Jacobs D., et al. In Laboratory and Test Handbook; Jacobs D.S; Kasten B.L., De Mott W.R., Wolfson W.L., Eds; Lexi-Comp Inc: Hudson (Cleveland), 1990; P.219.
6. Gordon T. et al.: Am. J. Med., 62; 707(1977).
7. Wiliams P., Robinson D., Baily A.: Lancet, 1/72(1979).
8. Goto A.M.: Hospital Practice, 23; Suppl., 1, 4(1988).
9. Crouse J.R. et al.: J. Lipid Res., 26; 566(1985).
10. Badmion J.J., Badmion L., Fuester V.: Journal of Clinical Investigation, 85:1234-41(1990).
11. Castelli W.P. et all.: Circulation, 55; 767(1977).
12. Barr D.P., Russ E.M., Eder H.A.: Am. J. Med., 11; 480(1951).
13. Kannel W.B., Castelli W.P., Gordon T.: Ann. Intern. Med., 90:85 (1979).

SYSTEM PARAMETERS

Method	End Point
Wavelength	578 nm
Zero Setting	Reagent Blank
Temperature Setting	37° C
Incubation Temperature	37° C
Incubation Time	5 min + 5 min
Delay Time	----
Read Time	----
No. of Reading	----
Interval Time	----
Sample Volume	0.005 ml (5 ul)
Reagent Volume	0.6 ml (600 ul)
Calibrator Concentration	Refer Calibrator vial
Units	mg / dl
Factor	----
Reaction Slope	Increasing
Linearity	180 mg / dl

IVD

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