HDL CHOLESTEROL KIT

(PEG / CHOD - PAP method)

For the determination of HDL Cholesterol
in serum or plasma.
(For Invitro Diagnostic Use Only)

Summary

Lipoproteins are the proteins which mainly transport fats in the blood stream. They can be grouped into chylomicrons, very low density lipoproteins (VLDL), low density lipoproteins (LDL) and high density lipoproteins (HDL). Chylomicrons and VLDL transport mainly triglycerides, though VLDLs also transport some amount of cholesterol. LDL carries cholesterol to the peripheral tissues where it can be deposited and increase the risk of arteriosclerotic heart and peripherial vascular disease. Hence high levels of LDL are atherogenic. HDL transports cholesterol from the peripherial tissues to the liver for excretion, hence HDL has a protective effect. The measurement of total and HDL cholesterol and triglycerides provide valuable information for the risk assessment of coronary heart diseases.

Principle

When the serum is reacted with the Polyethylene Glycol contained in the precipitating reagent, all the VLDL and LDL are precipitated. The HDL remains in the supernatant and is then assayed as a sample for cholesterol using the Cholesterol (CHOD/PAP) reagent.

Normal reference values

		Prognostically favourable	Standard Risk Level	Risk Indicator
HDL Chol. (mg/dl)	males	> 55	35 - 55	< 35
	females	> 65	45 - 65	< 45
LDL Chol. (mg/dl)	males / females	< 150	150 - 190	> 190
Total Chol.	males	> 3.8	3.8 - 5.9	< 5.9
HDL Chol.	females	> 3.1	3.1 - 4.6	< 4.6

It is recommended that each laboratory establish its own normal range representing its patient population.

Contents	**75 ml**
L1 : Enzyme Reagent 1	60 ml
L2 : Enzyme Reagent 2	15 ml
L3 : Pricipitating Reagent	2.5 ml
S : HDL Cholesterol Standard (25 mg/dl)	5 ml

Storage / stability

Contents are stable at 2-8^0 C till the expiry mentioned on the labels.

Reagent Preparation

Reagents are ready to use.

Working reagent : Pour the contents of 1 bottle of L2 (Enzyme Reagent 2) into 1 bottle of L1 (Enzyme Reagent 1).This working reagent is stable for at least 8 weeks when stored at 2-8^0 C. Upon storage the working reagent may develop a slight pink colour however this does not affect the performance of the reagent.

Alternatively for flexibility as much of working reagent may be made as and when desired by mixing together 4 parts of L1 (Enzyme Reagent 1) and 1 part of L2 (Enzyme Reagent 2). Alternatively 0.8 ml of L1 and 0.2 ml of L2 may also be used instead of 1 ml of the working reagent directly during the assay.

Sample material

Serum, EDTA plasma. Cholesterol & HDL Cholesterol are reported to be stable in serum for 7 days when stored at 2-8^0 C.The sample should preferably be of 12 to 14 hours fasting.

Procedure

Wavelength / filter	:	505nm (Hg 546 nm) / Green
Temperature	:	37^{0}C / R.T.
Light path	:	1 cm

Precipitation of VLDL & LDL :

Pipette into a clean dry test tube :

Precipitating Reagent (L3)	0.1 ml
Sample	0.1 ml

Mix well and incubate at R.T. for 5 min. Centrifuge at 2500 - 3000 rpm to obtain a clear supernatant.

Cholesterol Assay :

Pipette into clean dry test tubes labelled as Blank (B), Standard (S), and Test (T) :

Addition Sequence	**B** (ml)	**S** (ml)	**T** (ml)
Working reagent	1.0	1.0	1.0
Distilled water	0.05	-	-
HDL Standard (S)	-	0.05	-
Supernatant *	-	-	0.05

Mix well and incubate at 37^{0}C for 5 min. or at R.T. (25^{0}C) for 15 min. Measure the absorbance of the Standard (Abs.S), and Test Sample (Abs.T) against the Blank, within 60 Min.

* If only Total Cholesterol is to be determined use only 0.01 ml of D.W./Chol. Std./Sample directly in the cholesterol assay.

Calculations

$$\text{HDL Cholesterol in mg/dl} = \frac{\text{Abs.T}}{\text{Abs.S}} \times 25 \times 2$$

(Where 2 is the dilution factor due to the deprotenization step)

Calculation of LDL Cholesterol (mg/dl) :

(Freidewald's Formula)

$$= \text{(Total cholesterol)} - \left(\frac{\text{Triglycerides}}{5}\right) - \text{(HDL Cholesterol)}$$

Freidewald's Formula is reliable provided that :
1. No chylomicrons are present i.e. it is a fasting sample.
2. Triglyceride values are below 400 mg/dl.
3. Type III hyperlipoproteinemia is absent.

Linearity

This procedure is linear upto 150 mg/dl of HDL Cholesterol. If values exceed this limit, dilute the serum with normal saline (NaCl 0.9%) and repeat the assay. Calculate the value using the proper dilution factor.

Note

The supernatant should be clear. If it is hazy or cloudy, the sample should be diluted 1 + 1 with normal saline (NaCl 0.9%) and the precipitation step should be repeated. (Results x 2)
Anticoagulants such as flouride, oxalates and hemolysed serums should not be used.

References

Trinder, P., (1969) Ann. Clin. Biochem. 6 : 24
Allain, C.C., et al, (1974) Clin. Chem. 20 : 470
Flegg, H.M., (1972) Ann. Clin. Biochem. 10 : 79
Grillo, F., et al, (1981) Clin. Chem. 27 : 375
Demacker, P.N.M., et al, (1980) Clin. Chem. 26 : 1775

System Parameters

Reaction	:	End Point
Wavelength	:	505 nm
Zero Setting	:	Reagent Blank
Incub. Temp.	:	37°C / R.T.
Incub. Time	:	5 min. / 15 min.
Delay Time	:	---
Read Time	:	---
No. of read.	:	---
Interval	:	---
Sample Vol.	:	0.05 ml
Reagent Vol.	:	1.00 ml
Standard	:	25 mg/dl x 2
Factor	:	---
React. Slope	:	Increasing
Linearity	:	150 mg/dl
Units	:	mg/dl

HDL(Kit).01(P)

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