HDL-CHOLESTEROL(PTA)

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(PTA- METHOD)

Reagent kit for quantitative estimation of high density lipoprotein (HDL) cholesterol in serum or plasma.

BACKGROUND & SYNOPSIS :

The common classification of lipoprotein (a) high density (HDL) (b) low density (LDL) (c) very low density (VLDL) comes mainly from ultracentrifugation of serum or plasma. As the word indicates, it is based on the density of lipoproteins. Chylomicrons are formed from lipid and protein associations giving opalescent appearance to the plasma. These are even lighter than very low density lipoproteins.

High density lipoproteins (specific gravity more than 1.063) can be separated by using polyionic substances along with bivalent metal ion. Cholesterol distribution in the different fractions of lipoproteins i.e. HDL, LDL, VLDL and chylomicrons is of particular interest in understanding the metabolic status and risk of various diseases like artherosclerosis, coronary heart disease (CHD), etc.

PRINCIPLE :

High density lipoproteins (HDL) are separated from other lipoprotein fractions by treating serum with phosphotungstic acid and magnesium chloride. HDL remains in solution while all other lipoprotein fractions are precipitated; cholesterol content of which is estimated by enzymatic method.

Serum + PTA Reagent ⟶ Supernatant (HDL) + Precipitates (other fractions)

DIAGNOSTIC SIGNIFICANCE :

Cholesterol from liver is transported to various tissue cells by low density lipoproteins, very low density lipoproteins and chylomicrons through blood (plasma) circulation. Cholesterol is also synthesized by the cells as needed. The excess cholesterol is transported back to liver by HDL. HDL therefore indicates excess cholesterol received by liver, where it is converted to bile salts and excreted in bile. Elevated levels of HDL-Cholesterol suggest a balanced status of cholesterol metabolism in tissues. Lower levels of HDL cholesterol are associated with higher risks of artherosclerosis (i.e. deposition of cholesterol in cells of blood vessels) and complications like hypertension, coronary heart disease (CHD) related to it.

PRESENTATION :

All reagents to be stored at 2-8^0 C.	No. of bottles
• 3 HDL - CHOLESTEROL (Precipitating Reagent) Ready for use	1
• HDL - CHOLESTEROL STANDARD. (50 mg/dl.)	1

SPECIMEN COLLECTION :

Fresh, clear serum under fasting condition with no hemolysis is the specimen of choice. However, plasma collected using heparin as an anticoagulant may also be used.

SEPARATION OF HDL - FRACTION :

PIPETTE INTO TEST TUBES		TEST
• Sample	(ml)	0.2
• 3-HDL - CHOLESTEROL	(ml)	0.2

Mix well and centrifuge at 3500-4000 rpm for ten minutes. Separate the clear supernatant immediately and determine cholesterol content as follows:

NOTE :

Do not subject HDL - Cholesterol Standard (50 mg/dl) provided in the kit to separation procedure of HDL.

REACTION PARAMETERS :

- Type of Reaction : End Point
- Wavelength : 505 nm
- Flowcell temperature : 37^{0}C
- Sample Volume : 50 µl (0.05 ml.)
- Reagent Volume : 1.0 ml.
- Incubation Time : 10min. at 37^{0}C
- Factor (Std. Conc.x2) : (50x2) = 100 mg/dl or 238.1
- Light Path : 1.0 cm.
- Zero setting with : Reagent Blank

PROCEDURE :

For instrument using 1 ml. cuvette capacity.

PIPETTE INTO TEST TUBES		BLANK	STD	TEST
• Cholesterol Reagent	(ml)	1.0	1.0	1.0
• HDL Standard	(ml)	-	0.05	-
• Supernatant	(ml)	-	-	0.05

Mix well and incubate for ten minutes at 37^{o}C and read absorbance of test and standard against reagent blank at 505nm (500-540 nm Green filter).

For instrument using 2.5 ml/3 ml cuvette capacity.

PIPETTE INTO TEST TUBES		BLANK	STD	TEST
• Cholesterol Reagent	(ml)	1.0	1.0	1.0
• HDL Standard	(ml)	-	0.1	-
• Supernatant	(ml)	-	-	0.1
Mix well and incubate at 37oC for 20 minutes.				
• Dist. Water	(ml)	2.0	2.0	2.0

Mix well and read absorbance of test and standard against reagent blank at (500-540 nm, Green filter).

TEST RESULTS :

$$\text{Serum HDL-Cholesterol} = \frac{\text{Abs. of Test}}{\text{Abs. of std.}} \times \text{Conc. of std.} \times \text{dilution factor}$$

$$= \frac{\text{Abs. of Test}}{\text{Abs. of std.}} \times 50 \times 2$$

NORMAL VALUES/RISK INDICATIONS :

	M E N	WOMEN
• NORMAL LEVEL (mg/dl)	30-55	45-65
• RISK INDICATOR (mg/dl)	Less than 30	Less than 45

The values of LDL-Cholesterol can be calculated, if the value of triglycerides is known by using Friedewald's equation.

$$\text{LDL-Cholesterol} = \text{Total Chol} - \frac{(\text{HDL-Chol.} + \text{Triglycerides})}{5}$$

The formula is valid only if Triglyceride values are normal or not very high.

REFERENCE :

Burstein M. Scholnick, H.P. and Mortin, R Cholesterol in High Density lipoprotein : Using Mg^{++}/PTA; J. Lipid Res. 19. Pg. 583.

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