

HDL-CHOLESTEROL (PPT)

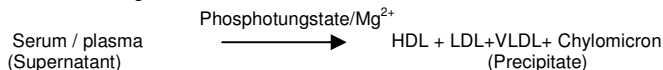
(Phosphotungstate Method)

Intended Use

HDL-Cholesterol - Precipitating reagent is used in conjunction with Cholesterol reagent kit for enzymatic determination of HDL-Cholesterol present in serum or plasma.

Principle

Phosphotungstate/ Mg^{2+} precipitates chylomicrons, LDL and VLDL fractions of lipids present in serum. High density lipoprotein (HDL) fraction remains unaffected in supernatant. Cholesterol content of HDL fraction is assayed using Cholesterol reagent kit.



Reagents Provided

1. HDL-Cholesterol - Precipitating reagent (Ready to use).
2. Standard - HDL Cholesterol (50 mg/dl).

Reagents and stability

The precipitating reagent and the standard are stable till the expiry date, if stored at $2^{\circ} - 8^{\circ}C$. Contamination of the reagent after opening the vials must be avoided.

Specimen collection and preservation

Blood should be collected in clean and dry container. Fasting blood is preferred for HDL-Cholesterol assay. Plasma should be separated immediately from the cells. For plasma separation any of the following anticoagulants may be used.

- EDTA 10 mg/ml of blood
- HEPARIN 200 IU/ml of blood

HDL-Cholesterol value is stable in serum for 24 hours, when stored at $2^{\circ} - 8^{\circ}C$ and 30 days, when stored at $-20^{\circ}C$

HDL SEPARATION

1. Take **equal amount** of serum/plasma (minimum 250 μ l) and HDL-Cholesterol precipitating reagent (minimum 250 μ l) in a centrifuge tube and mix it thoroughly.
2. Centrifuge it at 4000 rpm for 10 minutes in a common laboratory centrifuge to obtain a clear supernatant.

Assay guidelines for Analyzers

Reaction Type	End Point with standard
Reaction Slope	Increasing
Incubation Time	10 Min. at $37^{\circ}C$ / 30 min. at RT
Wave length	510 nm (505 - 530 nm)
Blank	Cholesterol Reagent
Sample volume	50 μ l (0.05 ml) Supernatant
Reagent Volume	1000 μ l (1.0 ml)
HDL Cholesterol Standard concentration	50 mg/dl
Factor Calculation	$(50 \text{ mg/dl} \div \text{Abs. of Std.}) \times 2$
Low Normal	30 mg/dl
High Normal	80 mg/dl
Linearity	Up to 200 mg/dl

Assay Guidelines for Manual Procedure

HDL Estimation with Cholesterol Liquid stable (LS) Reagent

Bring the Cholesterol reagent to room temperature and perform the assay as given below.

Assay the supernatant for HDL-Cholesterol within 2 hrs. after centrifugation using working solution of Cholesterol reagent.

Reagents	Blank	Standard	Sample
Cholesterol Reagent	1000 µl (1.0 ml)	1000 µl (1.0 ml)	1000 µl (1.0 ml)
Standard	-	50 µl (0.05 ml)	-
Supernatant	-	-	50 µl (0.05 ml)

1. Mix thoroughly and incubate the tubes at 37°C for 10 minutes or at room temperature for 30 minutes.
2. Read the absorbance against cholesterol reagent blank at 510 nm (505 - 530 nm).
3. The final colour is stable for 2 hours, if not exposed to direct light.

Calculation

$$\text{HDL-Cholesterol con.in sample (mg/dl)} = \frac{\text{Abs. of Sample} \times 100^*}{\text{Abs. of Standard}}$$

* Factor of 100 (not 50) is used for calculation due to serum dilution during precipitation step.

Friedwald's equation for LDL = Total cholesterol – [HDL+ (TGL ÷ 5)]

LDL - Low Density Lipoprotein.

HDL - High Density Lipoprotein.

TGL - Triglycerides.

Normal Range

Guidance value for Men : 30 - 60 mg/dl

Guidance value for Women : 33 - 80 mg/dl

Note: Expected range varies from population to population and each laboratory should establish their own normal range.

Limitation

This method is linear up to 200 mg/dl. For higher values dilute sample 1:2 with normal saline and perform the assay. Multiply final result by 2 to get the real value.

Quality Control

To ensure adequate quality control measures, it is recommended that each batch should include a normal and an abnormal commercial reference control serum. It should be realized that the use of quality control material checks both instrument and reagent functions together. Factors which might affect the performance of this test include proper instrument function, temperature control, cleanliness of glassware and accuracy of pipetting.

References

1. Castelli, W.P. Metabolic therapy, 6, 1 (1977).
2. Castelli, W.P. et al. Circulation, 55, 767 (1977).
3. Gordon, T. et al. Am. J. Med., 62, 707 (1977).

Symbols

IVD	In Vitro Diagnostics.		Caution.		Keep away from sun light.		Date of Manufacture.
LOT	Batch No.		Read Instructions.		Fragile.		Product Expiry Date.
CONT	Content.		Storage Temperature.		Keep Dry.		Manufactured By.
REF	Catalogue No.						



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