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

Reagent

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

Principle:

Phosphotungstate/Mg2+ 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.



REAGENT STORAGE & STABILITY:

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

SPECIMEN COLLECTION AND STORAGE:

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˚ - 8˚ C and 30 days, when stored at -20°C

HDL SEPARATION:

1. Take equal amount of serum/plasma (minimum 250 µl) and HDL-Cholesterol precipitating reagent (minimum 250 µl) 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.

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	1000 µl	1000 µl
Standard	-	50 µl	-
Supernatant	-	-	50 µl

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).

CALCULATIONS:

HDL-Cholesterol con.in sample = Abs. of Sample x 100*
(mg/dl) 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.

LIMITATION:

1. Reaction is linear up to 200 mg/dl. If the cholesterol value exceeds 1000 mg/dl, then dilute the specimen suitability with normal saline and repeat the assay. In such case the results obtained should be multiplied by dilution factor to obtain correct cholesterol value.
2. The standard is a viscous solution. Use broad mouth pipette for accurate pipetting.

ASSAY PARAMETERS:

Reaction Type	**End Point with standard**
Reaction Slope	**Increasing**
Incubation Time	**10 Min. at 37˚C / 30 min. at RT**
Wave length	**510 nm (505 - 530 nm)**
Blank	**Cholesterol Reagent**
Sample volume	**50 µl (0.05 ml) Supernatant**
Reagent Volume	**1000 µl (1.0 ml)**
Standard conc.	**50 mg/dl**
Factor Calculation	**(50 mg/dl ÷ Abs. of Std.) x 2**
Low Normal	**30 mg/dl**
High Normal	**80 mg/dl**
Linearity	**Up to 200 mg/dl**

LINEARITY:

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.

REFERENCE VALUE:

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.

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, and 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).
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