



HDL CHOLESTEROL (PHOSPHOTUNGSTIC ACID Method - End Point)

Intended Use

The reagents are used for the quantitative determination of HDL cholesterol in serum or plasma. For in-vitro diagnostic use only.

Introduction

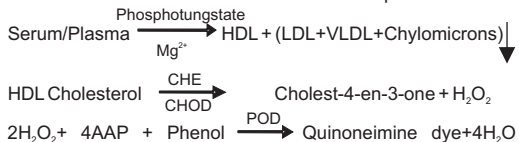
The principle role of HDL in lipid metabolism is the uptake and transport of cholesterol from peripheral tissues to the liver through a process known as reverse cholesterol transport. About thirty percent of blood cholesterol is carried by HDL; it is called good cholesterol (HDL-C). A high level of HDL-C seems to protect against cardiovascular disease and low levels of HDL cholesterol (less than 40 mg/dl) increase the risk of heart disease. The measurement of total cholesterol, HDL cholesterol and triglycerides provide valuable information concerning the prediction of coronary heart disease.

Method

Phosphotungstic acid method, End Point.

Principle

When serum is treated with phosphotungstate in the presence of magnesium ion, the LDL, VLDL and chylomicron are precipitated from serum. The HDL cholesterol remains dissolved in the supernatant. The supernatant then acts as a sample and assayed for cholesterol by an enzymatic method. For all abbreviations below refer to Cholesterol pack insert.



Absorbance of quinoneimine is directly proportional to the HDL cholesterol concentration, when measured at 505nm.

Reagent Composition

Reagent 1:

Phosphotungstic acid	2.045 mmol/l
Magnesium Chloride	39.35 mmol/l

Reagent 2:

HDL Cholesterol Standard	50 mg/dl
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*Cholesterol reagent is not provided with this kit.

Precautions

Following precaution should be taken:

- Avoid ingestion, do not pipette by mouth.
- Avoid contact with skin and eyes. If spilled, thoroughly wash affected area with water.
- Flush with plenty of water while disposing.

Reagent Storage and Stability

Unopened reagents are stable till expiry mentioned on the label when stored at 2–8°C. Standard Reagent 2 is stable till expiry as mentioned on the label when stored at 2–8°C.

Reagent Preparation

Reagents are ready for use.

Reagent Deterioration

Reagents should be clear. Turbidity and/or precipitation may be because of reagent deterioration.

Sample Collection and Storage

Unhaemolysed serum or plasma can be used for the testing. Anti-coagulants like EDTA and heparin can be used. Citrates should not be used as anti-coagulant. It is recommended to use freshly collected samples for assay. Serum samples can be stored for 7 days at 2–8°C and 3 months when frozen .

General Assay Parameters

Mode	End Point
Wavelength (nm)	505
Wavelength Range Usable (nm)	500-550
Blank with	Reagent
Sample Volume (µl)	25/50
Cholesterol Reagent (µl)	500/1000
Incubation Time (min)	10
Incubation Temperature (°C)	37
Normal Low (mg/dl)	30
Normal High (mg/dl)	80
Linearity (mg/dl)	Upto 150
Standard Conc. (mg/dl)	50
Factor	2
Units	mg/dl

PROCEDURE

	Volume
Sample	250 µl
Reagent 1	250 µl

Mix well and allow the reaction mixture to stand for 10 minutes at room temperature and then centrifuge for 10 minutes at 4000 rpm to get a clear supernatant. This supernatant is used as sample to get the HDL cholesterol concentration in the patient sample.

Measurement of HDL by Cholesterol Reagent

One reagent blank and one standard are sufficient for each assay series.

Pipette into test tubes:

Particulars	Blank	Standard	Sample
Cholesterol Reagent	1000 µl	1000 µl	1000 µl
Dist. Water	50 µl	-	-
Reagent 2	-	50 µl	-
Sample (supernatant)	-	-	50 µl

Mix well & incubate for 10 min at 37°C. Measure the absorbance of standard (A std) and sample (A sample) against reagent blank at 505 nm.

Calculation

HDL Cholesterol concentraion in the sample can be calculated using the following formula:

$$\text{HDL Cholesterol} = \frac{\text{Absorbance of Sample}}{\text{Absorbance of Standar}} \times \text{Conc. of Std.} \times 2 \quad (\text{mg/dl}) \quad (\text{mg/dl})$$

Example: If the absorbance of sample is 0.109 and the absorbance of standard is 0.229. The calculation shall be:

$$\frac{0.109}{0.229} \times 50 \times 2 = 47.59 \text{ mg/dl}$$

If the HDL-cholesterol concentration exceeds 150mg/dl, dilute the sample with normal saline and repeat the assay. The reportable results in this case shall be calculated by multiplying the results obtained with dilution factor.

Reference value

Serum
 Male : 30 - 75 mg/dl
 Female : 35 - 85 mg/dl

Reference range varies from population to population; therefore, each laboratory should establish its own normal range

Limitations

1. The reagent and sample volumes can be altered proportionately so that the sample: reagent, ratio remains same.
2. High level of bilirubin and ascorbic acid interferes with precipitation.

Quality Control

The patient results obtained for each batch can be validated by using normal and abnormal control sera with assayed values for HDL cholesterol.

Performance

Linearity Limit: 150mg/dl

Precision:

Within run

Control	Control 1	Control 2
No. of samples	20	20
Mean (mg/dl)	36.29	26.67
S.D.	0.44	0.46
C.V. %	1.21	1.72

Between run

Control	Control 1	Control 2
No. of samples	60	60
Mean (mg/dl)	36.08	26.96
S.D.	0.35	0.40
C.V. %	0.96	1.49

References

1. Tietz NW. Clinical guide to laboratory tests, 2nd ed. Saunders Co., 1991.
2. Reducing risk by raising HDL-cholesterol: the evidence. # European Heart Journal Supplements Vol 8 Suppl F p. F23-F29 http://eurheartjsupp.oxfordjournals.org/cgi/content/abstract/8/suppl_F/F23
3. <http://www.careurheart.com/heart-care/articles/raising-hdl-cholesterol-level.htm>
4. NCEP expert panel, Arch Inter Med (148), 26-69, (1988).
5. Young DS. Effects of drugs on clinical laboratory tests, 4th ed., AACCPress, 1995.

Pack Presentation

Product Code/ Catalogue No.	Pack Size*	Reagent 1	Reagent 2
KGHCH103.2.1	1x50ml	1x50ml	1x2ml
KGHCH103.2.2	2x50ml	2x50ml	1x2ml











* Pack size may vary on market demand.

Revision No: (Ver: KGHCH103.2/1)

Date of Issue: 1st April 2010

Symbols

Following symbols are used in the labeling of KeeGad kits:

	Catalogue No.		Batch No.
	CE Mark		Read instructions
	In Vitro Diagnostics		Storage temperature
	Expiry Date		Content
	Product Name		Manufactured By



Manufactured by:
KEE GAD Biogen Pvt. Ltd.
 A-8, Third Floor, Naraina Industrial Area,
 Phase-II, New Delhi-110028 (India)