VioCHEM HDL Cholesterol Precipitation Reagent

(PEG Precipitation Method)

(For Invitro Diagnostic Use Only)

INTENDED USE

HDL Cholesterol Precipitation Reagent is used for the determination of HDL Cholesterol in serum or plasma.

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 peripheral vascular disease. Hence high levels of LDL are atherogenic. HDL transports cholesterol from the peripheral 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.

EXPECTED 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. / HDL Chol.	Males	>3.8	3.8 - 5.9	< 5.9
	Females	>3.1	3.1 - 4.6	< 4.6

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

PRESENTATION

REF	705190005
Pack Size	5 ml

L1 Precipitating Reagent 5 ml
S HDL Cholesterol Standard (25 mg/dl) 3 ml

COMPOSITION

PEG 6000; Non reactive Stabilizers , Detergents and Preservatives.

STORAGE / STABILITY

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

REAGENT PREPARATION

Reagents are ready to use.

After the precipitation step Cholesterol reagent is required additionally for conducting the Cholesterol assay.

SAMPLE MATERIAL

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

SAMPLE WASTE AND DISPOSAL

Do not reuse the reagent containers, bottles, caps or plugs due to the risks of contamination and the potential to compromise reagent performance.

This product requires the handling of human specimens. It is recommended that all human sourced material are considered potentially hazardous and are handled in accordance with the OSHA standard on blood borne pathogens.

Appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.

Handle specimens, solid and liquid waste and test components in accordance with local regulations and NCCLS guidelines M29, or other published biohazard safety guidelines.

MATERIALS REQUIRED BUT NOT PROVIDED

Photometer analyzer with standard thermostatic cuvette holder, micropipette and appropriate laboratory equipment.

PROCEDURE

Precipitation of VLDL & LDL:

Pipette into a clean dry test tube:

Precipitating Reagent (L1)	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.

Procedure for the Cholesterol Assay:

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

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 Cholesterol Standard (S)	--	0.05	--
Supernatant	--	--	0.05

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

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 deproteinization 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.

QUALITY CONTROL

The following process is recommended for QC during the assay of HDL Cholesterol Precipitation Reagent. *Define and establish acceptable range for your laboratory.

1. Two levels of control (Normal and Abnormal) are to be run on a daily basis.
2. If QC results fall outside acceptance criteria, recalibration may be necessary.
3. Review QC results and run acceptance criteria following a change of reagent lot.

SPECIFIC PERFORMANCE CHARACTERISTICS

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.

Limit of detection:

The limit of detection for HDL Cholesterol is 0.5 mg/dl.

Interferences:

Criterion: recovery within ±10% of initial value.

Hemolysis: no significant interference upto an H index of 100 (approx. hemoglobin concentration: 100 mg/dL or 62.1 µmol/L).

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Precision:

Precision studies were performed with two controls using NCCLS protocol EP5-A. The results of the precision studies are shown below:

Sample	Within-run		Between-run		Total	
	Mean	CV%	Mean	CV%	Mean	CV%
Control 1	39.3	6.63	39.5	4.58	78.8	11.21
Control 2	25.92	7.58	26.28	4.86	52.2	12.44

Method comparison:

Comparative studies were done to compare our reagent with another commercial HDL Cholesterol Assay. No significant differences were observed. Details of the comparative studies are available on request.

NOTE

In vitro diagnostic reagent for laboratory and professional use only Not for medicinal use. The reagent contain sodium azide 0.1% as preservative. Avoid contact with skin and mucosa. On disposal flush with large quantities of water. Only clean and dry glassware must be used. 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 fluoride, oxalates and hemolysed serums should not be used. The reagent may be used in several automated analyzers. Instructions are available on request. Do not use turbid, deteriorated or leaking reagents.

REFERENCES

1. Trinder P., (1969) Ann. Clin. Biochem. 6:24.
2. Allain C.C., et. Al., (1974) Clin. Chem. 20:470.
3. Flegg H.M., (1972) Ann. Clin. Biochem. 10:79.
4. Grillo F., et. al. (1981) Clin. Chem. 27 : 375.
5. Demacker P.N.M. et. al. (1980) Clin. Chem. 26 : 1775.
6. Data on file: Coral Clinical Systems.

System Parameters

Reaction	: End Point	**Interval**	: ---
Wavelength	: 505 nm	**Sample Vol.**	: 0.05 ml
Zero Setting	: Reagent Blank	**Reagent Vol.**	: 1.00 ml
Incub. Temp.	: 37^0 C / R. T.	**Standard**	: 25 mg/dl x 2
Incub. Time	: 5 min. / 15 min.	**Factor**	: ---
Delay Time	: ---	**React. Slope**	: Increasing
Read Time	: —	**Linearity**	: 150 mg/dl
No. of read.	: ---	**Units**	: mg/dl

SYMBOL KEYS

Temperature Limitation	Consult Instructions for use	Date of Manufacture	L1 — Precipitating Reagent
Manufacturer	IVD — *In vitro* Diagnostic Medical Device	This side up	S — HDL Cholesterol Standard (25 mg/dl)
Use by	REF — Catalogue Number	LOT — Batch Number / Lot Number	PEG Precipitation — PEG Precipitation Method

Manufactured by:

Coral Clinical Systems

A Division of Tulip Diagnostics (P) Ltd.

Plot No. M 46, Phase III B, Verna Industrial Estate, Verna, Goa - 403 722, INDIA.

Regd. Office: Gitanjali, Tulip Block, Dr. Antonio Do Rego Bagh, Alto Santacruz, Bambolim Complex P.O., Goa - 403 202, INDIA.

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