HDL
Direct Method

Intended Use:

HDL Cholesterol test reagent/kit is a medical device intended for the estimation of HDL Cholesterol in serum or plasma.

Clinical Significance

High- Density Lipoproteins (HDL) are one of the major classes of plasma lipoproteins. They are composed of a number of heterogeneous particles, including cholesterol and vary with respect to size and content of lipid and apolipoprotein. HDL serves to remove cholesterol from the peripheral cells of the liver, where the cholesterol is converted to bile acids and excreted into theintestine.

An inverse relationship between HDL – Cholesterol (HDL-C) levels in serum and the incidence/prevalence of Coronary Heart Disease (CHD) has been demonstrated in a number of epidemiological studies.

The importance of HDL-C as a risk factor for CHD is now recognized. An accurate measurement of HDL-C is of vital importance whenassessing patient risk from CHD.

Principle

Specific and special detergent react with HDL-C fractions, followed by enzymatic reactions with CE, CO, POD and with Chromogenic coupler leading to form color, simultaneously non HDL-C Lipoproteins (CM,VLDL & LDL) form colorless water-soluble compound with other group of specific detergents. The HDL-C fraction reaction as per below:

HDL-Cholesterol Esters —CE→ Cholesterol+ fatty acids

Cholesterol +O2 —CO→ Cholesterol-4-en-3-One + H2O2

2H2O2 + 4AAP+ Phenolic compd. —POD→ Quinoneimine dye + 4H2O

Reagent Composition

R1 HDL Reagent	CHOD – 200U CE – 100U POD – 1000U Activators and stabilizers
HDL Calibrator	Refer vial label for conc.

Working Reagent Preparation

Reagent is ready to use.

HDL Calibrator Preparation & Stability

For reconstitution refer the Calibrator vial label.

After reconstitution Calibrator stable for 7 days at2-8ºC

Stability and Storage

Storeat 2-8ºC

All the kit contents are stable until the expiry date stated on thelabel. Do not use reagents beyond the expiration date.Store the vials tightly closed protected from light and preventcontaminations during the use.

Materials required

- Photometer or spectrophotometer with a thermostat cell compartment set at 25/30/37ºC, capable of reading at 620nm.
- Stopwatch, strip-chart recorder or printer.
- Cuvettes with 1-cm path length
- Pipettes to measure reagent and samples.

Sample and Stability

Serum or plasma

On empty stomach serum or heparinized plasma free of hemolysis. EDTA Plasma is acceptable but causes lower resultsdo not freeze the samples. If any sample show precipitation, centrifuge before using.

HDL reported to be stable in serum or plasma for 7days at 2-8ºC.

Assay Procedure

Pipette into clean dry test tubes labeled as, Blank (B), Standard (S) and Test (T):

Addition Sequence	Blank	Calibrator	Test
R1 HDL Reagent	400µl	400µl	400µl
Calibrator	-	5µl	-
Sample	-	-	5µl

Mix & Incubation for 5 mins at 37°C .Read Abs of Test (T) and Calibrator(C) against Reagent Blank at 620nm.

Calculation

$$\text{HDL(mg/dl)} = \frac{\text{Abs.of Test}}{\text{Abs.ofCalibrator}} \times \text{conc. of Calibrator}$$

Quality Control

To ensure adequate quality control (QC), each run should include aset of controls (normal and abnormal) with assayed values handledas unknowns.

If the values are found outside of the defined range, check the instrument, reagents and procedure.

Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable results.

Linearity

The procedure is linear up to 250 mg/dl. If values exceed this limit, dilute the sample with distilled water and repeat the assay. Calculate the value using the proper dilution factor.

Reference Values

Serum, Plasma

Male : 35–80mg/dl

Female : 42 – 88mg/dl

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

General System Parameters

Mode	End Point
Reaction	Increasing
Wavelength 1	620 nm
Wavelength 2	700 nm
Blank with	Reagent
Sample Volume	5µl
Reagent Volume	400µl
Std Conc.	Refer vial label
Incub. Temp.	37°C
Incub. Time	5 min.
Normal Range	35 – 88mg/dl
Linearity	Up to 250 mg/dl
Unit	mg/dl

Notes

1. A special surfactant, Lipid Clearing Factor (L.C.F.) is added to the Reagent to solubilise the lipemic sera (causing turbidity or opalescence) which adds to the accuracy of results.
2. Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.

Reference

1. National Institute of Health Consensus Development Conference Statement: Triglycerides, High Density Lipoprotein, and Coronary Heart Disease. Washington D.C. Feb26-28,1992.
2. Second Report of the Expert Panel on Detection, Evaluation, and Treatment of high Blood Cholesterol in Adults (Adult Treatment Panel II). NH Publication No. 93-3096, September1993.

For *in vitro* Diagnostic use only.