Pariksha Biotech
A game changer in IVD

Command on Quality

High-Q HDL Cholesterol - Direct (PVS- PEGME Method 5 th Gen)

Intended Use: Kit for the quantitative determination of HDL-Cholesterol concentration in human serum and plasma by direct method

Summary:
Plasma lipoproteins are spherical particles containing varying amounts of cholesterol, triglycerides, phospholipids and proteins. The phospholipid, free cholesterol and protein constitute the outer surface of the lipoprotein particle, while the inner core contains mostly esterified cholesterol and triglyceride. These particles serve to solubilize and transport cholesterol and triglyceride in the bloodstream. The relative proportions of protein and lipid determine the density of these lipoproteins and provide a basis on which to begin their classification.The classes are: chylomicron, very low-density lipoprotein (VLDL), low-density lipoprotein (LDL) and high-density lipoprotein (HDL). Numerous clinical studies have shown that the different lipoprotein classes have very distinct and varied effects on coronary heart disease risk. 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 (a proposed cardio protective mechanism). Low HDL-C levels are strongly associated wth an increased risk of coronary heart disease and coronary artery disease. Hence, the determination of serum HDL-C is a useful tool in identifying high-risk patients. The Adult Treatment Panel of the National Cholesterol Education Program (NCEP) recommends that in all adults 20 years of age and over, a fasting lipoprotein profile (Total cholesterol, LDL cholesterol, HDL cholesterol and Triglycerides) should be obtained once every five years to screen for coronary heart disease risk. The reference method for the quantification of HDL-C combines ultracentrifugation and chemical precipitation to separate HDL from other lipoproteins, followed by cholesterol measurement by the Abell-Kendall method. The first routine methods widely utilized by laboratories involved selective precipitation and removal of LDL and VLDL, followed by the enzymatic measurement of HDL-C in the supernatant fraction. Since these methods require off-line pretreatment and separation steps the assay procedures cannot be fully automated. As a result, routine determination of HDL-C has suffered from long handling times and poor reproducibility.

Principle:
The assay is based on a modified polyvinyl sulfonic acid: (PVS) and polyethylene-glycol-methyl ether (PEGME) coupled classic precipitation method with the improvements in using optimized quantities of PVS/PEGME and selected detergents. LDL, VLDL, and chylomicron (CM) react with PVS and PEGME and the reaction results in inaccessibility of LDL, VLDL and CM by cholesterol Oxidase (CHOD) and cholesterol esterase (CHER). The enzymes selectively react with HDL to produce H2O2 which is detected through a Tinder reaction

$$HDL + LDL + VLDL + CM \xrightarrow{PVS} HDL + (LDL + VLDL + CM) \cdot PVS/PEGME$$

$$HDL + CHOD + CHER \xrightarrow{PEGME} Fatty\ Acid + H2O2$$

$$H2O2 + 4\text{-}AA + TODB \xrightarrow{Peroxidase} Quenone + 5\ H2O\ (\lambda max = 560nm)$$

Reagent Composition:
Reagent 1. Pipes Buffer (pH 7.0), TODB N,N-Bis(4-sulfobutyl)-3-methylaniline, Polyvinyl Sulfonic acid, Polyethylene-glycol-methyl ether, $MgCl_2$, detergent, EDTA.

Reagent 2. Pipes Buffer (pH 7.0), Cholesterol Esterase, Cholesterol Oxidase, Peroxidase, 4-Aminoantipyrine, Detergent.

HDL C Calibrator: Concentration value is traceable to NIST SRM1951b. **Add 1 ml distilled water to reconstitute**. Wait for 30 Minutes at Room temperature until it dissolves. Swirl gently to get the homogenous mixture. Calibrator is stable for 30 Days at 2-8°C and 3 Months when stored frozen as aliquots at -20°C

Reagent Presentation:
Reagent-1 and Reagent-2 are Liquid Stable and Ready to use. Calibrator needs to be reconstituted in distilled water.

Storage and Stability:
All unopened reagents are stable until the expiration date on the label when stored at 2-8°C.

Specimen Collection and Preparation:
Serum, EDTA-treated or heparinized plasma drawn from the patient after a 12 – 14 hour fast are the required specimens.

Serum: Collect whole blood by veni puncture and allow to clot. Centrifuge and remove the serum as soon as possible after collection (within 3 hours).

Plasma: Specimens may be collected in EDTA or lithium or sodium heparin. Centrifuge and remove the plasma as soon as possible after collection (within 3 hours).Serum or plasma should not remain at 15-30°C longer than 14 hours. If assays are not completed within 14 hours, serum or plasma should be stored at 2-8°C for up to 1 week. If specimens need to be stored for more than 1 week, they may be preserved at less than -70°C for up to 3 months. Samples may be frozen once. Refer to NCCLS Document H18-A for further instructions on specimen collection, handling, and storage.

Assay Procedure:
High-Q HDL-Cholesterol reagent is intended for measurement of serum HDL-Cholesterol using a clinical chemistry analyzer. Below is the best suited test procedure for many of the major analyzers. This procedure can be adapted and modified for use with other analyzers also.

Assay Procedure:
System Parameters

Reaction Type (Mode)	End Point
Reaction Direction	Increasing
Wave Length	546 nm /630 nm (Bichromatic)
Flow Cell Temp.	37°C
Blank	Reagent Blank
Reagent Volume	600 µl (R1) + 200 µl (R2)
Sample	8 µl
Calibrator Concentration	— (On the Vials Lot Specific)
Linearity	200 mg/dl

Reagent	Blank	Calibrator	Test
HDL R1	600 µl	600 µl	600 µl
Calibrator	----	8 µl	----
Sample	----	----	8 µl
Mix and Incubate for 5 Minutes at 37 °C			
HDL R2	200 µl	200 µl	200 µl

Mix well incubate for 5 minutes at 37°C

Mix and read absorbance of Calibrator (C) and Test (T) against Reagent Blank (B) at 546/630 nm bichromatically

(If the end user does not have two filters in the analyzer he can perform the assay at 546 nms alone monochromatically)

Calculations:

$$\text{HDL Concentration in mg/dl} = \frac{\text{Abs. of Test}}{\text{Abs. of Calibrator}} \times \text{HDL Calibrator Concentration (Lot Specific)}$$

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Measuring Range :
Is from detection limit of 3.0 mg/dl to linearity limit of 200 mg/dl. If the result obtained is greater than linearity limit, dilute the sample 1/2 with NaCl (9 g/L) and multiply the result by 2.

Calibration:
1) The High-Q HDL Cholesterol Calibrator is required for the calibration of this assay.
2) Other commercially available HDL calibrators have not been tested with this assay.
3) The value of the High-Q HDL Cholesterol Calibrator was assigned by procedures traceable to National Reference System for Cholesterol (NRS/CHOL).
4) Since different methods of HDL-estimation Reagents are available now a days, the concentration of High-Q HDL-C calibrator must not be cross compared with other commercially available calibrators as all the calibrator manufacturers' fix the calibrator concentration depending upon the type of HDL-Cholesterol Assay they are using for calibration of calibrators at their end

Calibration Frequency :
Recalibration is recommanded.
• Whenever the reagent lot is changed
• as per the requirement of QC procedures.

Performance Characteristics:
1. Reagent Blank : < 0.200 Abs.
2. Specificity : 90 - 110 % of expected values
3. Reproducibility : Coefficient of variation < 5 % (Within run and in between runs)

Expected Values:
The following NCEP cutpoints for patient classification are used for the prevention and management of coronary heart disease It is recommended that each laboratory should verify the reference interval for its patient population.

Males:	**30 - 70 mg/dL**
Females:	**30 - 85 mg/dL**

Accuracy :
Results obtained using High-Q HDL reagents (y) did not show systematic difference when compared with another commercial reagents (x).
The results obtained using 92 samples were the following :
Correlation coefficient : 0.998.
Regression equation: y=4.6 + 0.940 (x)
The results of the performance characteristics depend on the analyzer used.

Quality Control:
Reliability of test results should be routinely monitored with quality-control materials or serum that reasonably represent performance with patient specimens. Controls or serum pools should be run with each assay to ensure that the reagents are functioning properly. An acceptable range for each lot of control material should be established by the laboratory.

Limitations / Interfering Substances:
All interference studies were conducted .

Substance Tested	Concentration with no significant (±10%) interference
Ascorbic Acid	120 mg/dL
Hemoglobin	1100 mg/dL
Bilirubin	50 mg/dL
Gamma-Globulins	7000 mg/dL
Lipemia as Triglycerides	2000 mg/dL

Ordering Information:

Ref./Cat. No.	Pack Size	Presentation
P-HDL-40	40 ml	30 ml (R1) + 10 ml (R2)
P-HDL-80	80 ml	2 x 30 ml (R1) + 2 x 10 ml (R2)
P-HDL-160	160 ml	2 x 60 ml (R1) + 2 x 20 ml (R2)
P-HDL-320	320 ml	4 x 60 ml (R1) + 4 x 20 ml (R2)

Product Features

- **Liquid Stable, Ready to use, Two Reagents. (3 Parts R1+ 1 Part R2)**
- **10 Minutes Assay**
- **Linearity 200 mg/dl**
- **Results Correlate with other Reference Methods.**
- **Meets NCEP Guidelines.**
- **Works well with Fasting & Non fasting patient samples.**
- **Precision with high triglyceride samples.**
- **Measuring Wavelength 546 nm (Monochrmatic), 546/630 nm (Bichromatic)**
- **Lyophilized Calibrator provided**
- **Serum / Heparinized or EDTA Plasma as Specimens**
- **Available as multi purpose reagents and system packs**

Symbols used with IVD devices

	Date of manufacture		Manufactured by
IVD	In vitro diagnostic device		Keep away from sunlight
	Do not freeze		This way up
	Use by (yyyy-mm-dd or mm/yyyy)	R	Reagent
CAL	Calibrator Material	LOT	Batch code
2°C 8°C	Temperature limitation (store at)	CONTROL	Control
	Consult instructions for use		Keep dry Keep away from rain
REF	Catalog Number		

eIFU Indicator

Pariksha's world inside
SCAN TO EXPLORE MORE

Manufactured in India by :
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