# LiquiMAX HDL Cholesterol - Direct

## (PVS- PEGME Method 5 th Gen)

**ORDERING INFORMATION**

| Ref. No. | Pack Size | Presentation |
|---|---|---|
| AVHDL2 - 40 | 40 ml | 30 ml (R1) + 10 ml (R2) |
| AVHDL2 - 80 | 80 ml | 2 x 30 ml (R1) + 2 x 10 ml (R2) |
| AVHDL2 - 160 | 160 ml | 2 x 60 ml (R1) + 2 x 20 ml (R2) |
| AVHDL2 - 320 | 320 ml | 4 x 60 ml (R1) + 4 x 20 ml (R2) |

**INTENDED USE:**
LiquiMAX HDL Cholesterol - Direct is an in-vitro diagnostic kit for the quantitative determination of HDL- Cholesterol concentration in human serum and plasma.

**PRODUCT FEATURES:**
1. Liquid Stable, Ready to use, Two Reagents. (3 Parts R1+ 1 Part R2)
2. 10 Minutes End Point Assay
3. Linearity 200 mg/dl
4. Results Correlate with other Reference Methods.
5. Meets NCEP Guidelines.
6. Works well with Fasting & Non fasting patient samples.
7. Precision with high triglyceride samples.
8. Measuring Wavelength 546 nm (Monochromatic), 660/546 nm (Bichromatic)
9. Lyophilized Calibrator provided
10. Serum / Heparinized or EDTA Plasma as Specimens
11. Multi Purpose Reagents and System Packs

**CLINICAL SIGNIFICANCE :**
Plasma lipoproteins are spherical particles containing varying amounts of cholesterol, triglycerides, phospholipids and proteins. The phospholipids, 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 with 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 triglyceride) 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

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

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

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

**STORAGE AND STABILITY:**
All unopened reagents are stable until the expiration date on the label when stored at 2-8°C.

**KIT COMPONENTS**
1. Buffer Reagent R1
2. Substrate Reagent R2
3. HDL Calibrator : Concentration as stated on the label

**COMPOSITION:**
**Reagent 1.** MES buffer (pH 6.5), TODB N,N-Bis(4-sulfobutyl)-3-methylaniline, polyvinyl sulfonic acid, polyethylene-glycol-methyl ether, MgCl2, detergent, EDTA.

**Reagent 2.** MES buffer (pH 6.5), cholesterol esterase, cholesterol oxidase, peroxidase, 4-aminoantipyrine, detergent.

**HDL C Calibrator:** Concentration value is traceable to NIST SRM1951b

**REAGENT RECONSTITUTION & STABILITY**
Reagent-1 and Reagent-2 are Liquid Stable and Ready to use. Calibrator needs to be reconstituted in distilled water.

**MATERIAL REQUIRED BUT NOT PROVIDED**
Laboratory Instrumentation, Spectrophotometer UV/VIS with thermostatic cuvette holder or clinical chemistry analyzer: semi auto, calibrated micropipettes, glass or high quality polystyrene cuvettes, test tube/rack, heating bath controls, saline.

**REAGENT DETERIORATION**
Discard any turbid reagent if blank reagent absorbance exceeds 0.2 at 546 nm against distilled water.

**WARNING & PRECAUTIONS**
- Reagent may contain some non reactive and preservative components. It is recommended to handle carefully, avoiding contact with skin and ingestion.
- Specimen should be considered infectious and handled appropriately.
- Contamination by soap or glycerol will affect this assay.
- Perform the test according to the general " Good Laboratory Practice" GLP

**SPECIMEN COLLECTION AND STORAGE**
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 venipuncture 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.

## SYSTEM PARAMETER

| | | |
|---|---|---|
| Reaction Type | : | End Point |
| Wave Length | : | 546 nm |
| Flow Cell Temp. | : | 37 °C |
| Reagent Volume | : | R1-600 µl+ R2-200 µl |
| Sample Volume | : | 8 µl |
| Blanking with | : | Reagent |
| Calibrator Concentration | : | Printed On the Label |
| Unit | : | mg/dl |
| Low Normal | : | 30 |
| High Normal | : | 70(Male), 85(Female) |
| Linearity | : | 200 |

## TEST PROCEDURE:

| Reagent | Blank | Calibrator | Test |
|---|---|---|---|
| Reagent R1 | 600 µl | 600 µl | 600 µl |
| Calibrator | — | 8 µl | ---- |
| Sample | — | — | 8 µl |
| Mix and incubate at 37 °C for 5 minutes | | | |
| Reagent R2 | 200 µl | 200 µl | 200 µl |

**Mix and incubate at 37 °C for 5 minutes.**
Read absorbance calibrator and Test against Blank at 546 nm.

## CALCULATION:

$$\text{HDL Conc. In Serum or Plasma} = \frac{\text{Abs. of Test}}{\text{Abs. of Calibrator}} \times \text{Calibrator Conc.}$$

## 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

## QUALITY CONTROL & CALIBRATION

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.

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

## PERFORMANCE CHARACTERISTICS

**1. Linearity**
Linearity : 200 mg/dl

**2. Sensitivity/ Limit of Detection (LOD)**
The lower limit of detection is 3.7 mg/dl

**3. Interferences**
All interference studies were conducted .

| Substance Tested | Concentration with no significant (±10%) Interference |
|---|---|
| Ascorbic Acid | 100 mg/dL |
| Hemoglobin | 1000 |
| Mg/dL Bilirubin | 60 mg/dL |
| Gamma-Globulins | 5000 |
| Mg/dL Lipemia as Triglycerides | 1800 mg/dL |

**4. Precision:**
Reproducibility was determined using controls. The following results were obtained:

**Intra-Assay**

| Sample | Mean (mg/dl) | SD (mg/dl) | % CV |
|---|---|---|---|
| Control 1 | 45.1 | 1.35 | 1.32 |
| Control 2 | 60.2 | 0.98 | 0.62 |
| Control 3 | 120.5 | 0.99 | 0.55 |

**Inter-Assay**

| Sample | Mean (mg/dl) | SD (mg/dl) | % CV |
|---|---|---|---|
| Control 1 | 45.2 | 1.71 | 1.38 |
| Control 2 | 60.3 | 3.13 | 1.93 |
| Control 3 | 120.8 | 1.97 | 1.06 |

**5. Method Comparison:**
Results obtained using LiquiMAX 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.

## LIMITATIONS

From detection limit of 3.7 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.

## WASTE DISPOSAL

Reagents must be disposed off in accordance with local regulations.

## REFERENCE


1. Williams Pet al.,High density lipoprotein and coronary risk factor, Lancet. 1:72 (1979)
2. Gordon, T.Castelli,W.P Hjortland, M.C et al.Am.J.Med.62, 707-714 (1977)
3. Rifai, N.and Warnick, G.R., Ed. Laboratory Measurement of Lipids,Lipoproteins and Apolipoproteins AACC Press. Washington,DC, USA,1994.


## Symbols Used on Pack

| | | | |
|---|---|---|---|
| REF | Catalogue Number | | Warning/Caution |
| LOT | Batch No. | IVD | In vitro diagnostic device |
| | Manufacturing Date | | Storage Limit |
| | Expiry Date | | Consult instruction for use |
| | Manufacturer | | Keep away from sunlight |
| | Keep Dry | | Do not use if package is damaged |
| | Contains sufficient no. of test | | |

ISO 13485 Certified Company

5 Schedule MDR 2017


Manufactured in India by :
AVECON Healthcare Pvt. Ltd.
Transforming Research into Innovations
Plot No.: 338, Sector-2, Industrial Growth Centre, Saha, Ambala, Haryana (INDIA)-133104
E-mail : helpdesk@aveconhealthcare.com, Website : www.aveconhealthcare.com
Customer Care No. : +91 93065 12576, CIN No.: U24230HR2006PTC118875



Ver. : 05/01-26