HDL CHOLESTEROL

Direct Reagent Kit

Liquid Reagent

INTENDED USE:

This reagent kit is used for *in-vitro* quantitative determination of HDL Cholesterol in human serum and plasma.

TEST PRINCIPLE:

The reaction between cholesterol other than HDL & enzyme for cholesterol assay is suppressed by the electrostatic interaction between polyanions & cationic substances. Hydrogen peroxide is formed by the free cholesterol in HDL by cholesterol oxidase. Oxidative condensation of EMSE and 4-AA is caused by hydrogen peroxide in the presence of peroxidase, and the asorbance of the resulting red-purple quinine is measured to obtain the cholesterol value in HDL.

REACTION :

Other lipoprotein than HDL $\xrightarrow{\text{Polyanions}}$ Suppress reaction with enzyme

HDL (Cholesterol esters) + H_2O $\xrightarrow[\text{Cholesterol Esterase}]{\text{Cationic substances}}$ HDL (Free Cholesterol) + Free fatty acids

HDL (free cholesterol) + O_2 + H^+ $\xrightarrow{\text{Cholesterol Oxidase}}$ Cholestenone + H_2O_2

$2H_2O_2$ + 4-AA + EMSE + H_3 + O $\xrightarrow{\text{Peroxidase}}$ Red-Purple quinine + $5H_2O$

KIT CONTENTS:

Reagent 1 : R1 Reagent

Reagent 2: R2 Reagent

Reagent 3: Direct HDL Calibrator (Separately Provided)

Product Insert : 01 No.

PREPARATION OF REAGENT & STABILITY:

The Reagent 1 & Reagent 2 are ready to use.

Calibrator : Reconstitute with distilled water (Volume mentioned on calibrator vial label). Let it stand for 30 minutes at room temperature. Dissolve the content of the vial swirling gently to avoid the for mation of foam.

STORAGE AND STABILITY:

Reconstituted calibrator is stable only for 7 days at 2 - 8°C.

SPECIMEN COLLECTION AND STORAGE:

Fresh Serum (Free of Hemolysis).

PRECAUTIONS:

1. Storage conditions as mentioned on the kit to be adhered.
2. Do not freeze or expose the reagents to higher temperature as it may affect the performance of the kit.
3. Before the assy bring all the reagents to room temperature.
4. After use store the kit contents immediately as 2-8°C.
5. Avoid contamination of the reagent during assay process.
6. Use clean glassware free from dust or debris.

PROCEDURE (Automated):

Refer to specific instrument application instructions.

TEST PROCEDURE (Manual):

Pipette into clean dry test tubes labeled Blank (B), Calibrator (C) and Test (T) as follows:

Addition sequence	B	C	T
R1 Reagent	450 µl	450 µl	450 µl
Calibrator	-	5 µl	-
Sample	-	-	5 µl
Mix and Incubate for 5 min. at 37°C.			
R2 Reagent	150 µl	150 µl	150 µl

Mix and incubate for 5 min. at 37°C. Measure the absorbance of calibrator & Test against reagent blank at 578 nm.

CALCULATIONS:

$$\text{HDL-C Conc.} = \frac{\text{Abs of Test}}{\text{Abs of Calibrator}} \times \text{Calibrator Conc.}$$

NORMAL VALUES*:

Male : 35 to 80 mg/dl

Female: 42 to 88 mg/dl

Each Laboratory should establish it's own normal range representing its patient population.

LINEARITY :

This procedure in linear upto 150 mg/dl. If the values exceed this limit, dilute the sample with normal saline (NaCl 0.9 %) and repeat the assy. Multiply result by dilution factor.

CLINICAL SIGNIFICANCE:

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 excreation, 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.

APPLICATIONS:

Input parameters for semi- auto / auto analyzers are given below:

INPUT PARAMETERS	VALUES
Reaction type	End point
Wave length	578 nm (578 - 620 nm)
Temperature	37°C
Incubation	5 min. + 5 min.
Reagent volume	R1 450 µl + R2 150 µl
Sample volume	5 µl
Zero setting	Deionised water
Light path	1.0 cm
Unit	mg/dl
Linearity	150 mg/dl
Calibrator Conc.	As Indicated on the Vial Label

QUALITY CONTROL:

For accuracy, it is necessary to run known controls with every assay.

REFERENCES:

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

Manufactured by:
Vanguard Diagnostics Private Limited
C-123, Phase-I, Okhla Industrial Area
New Delhi-110 020, INDIA.

Tel: 011-4172 7222,
Email:info@vanguarddiagnostics.com
Website : www.vanguarddiagnostics.com

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