LIQUIZYME

DIRECT HDL CHOLESTEROL

(PEGME Method)

BEACON

Code	Product Name	Pack Size
LS021A	Liquizyme Direct HDL Cholesterol	40 ml
LS021B	Liquizyme Direct HDL Cholesterol	80 ml
LS021C	Liquizyme Direct HDL Cholesterol	160 ml
LS021D	Liquizyme Direct HDL Cholesterol	320 ml

Intended Use

Diagnostic reagent for quantitative *in vitro* determination of HDL Cholesterol in human serum and plasma.

Clinical Significance

High-density lipoproteins (HDL) compose one of the major classes of plasma lipoproteins. They are synthesized in lilver as complexes of apolipoprotein and phospholipid and are capable of picking up cholesterol and carring it from arteries to the liver, where the cholesterol is converted to bile acids and excreted into the intestine.

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.

Accurate mesurement of HDL-C is of vital importance when assessing patient's risk for CHD.

Principle

The assay is based on a modified polyvinyl sulfonic acid (PVS) and polyethyleneglycol-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 inaccessibililty of LDL, VLDL and CM by cholesterol oxidase (CHOD) and cholesterol esterase (CHER).

The enzymes selectively react with HDL to produce H_2O_2 which is detected through a Trinder reaction.

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

$$\text{HDL} \xrightarrow{\text{CHOD, CHER}} \text{Fatty Acid + } H_2O_2$$

$$2H_2O_2 + \text{4-AA + TODB} \xrightarrow{\text{Peroxidase}} \text{Quinone + } 5H_2O$$

Reagent Composition

Reagent 1 : HDL R1 Reagent

Buffer	:	> 5 mmol/l
$MgCl_2$	:	> 2 mmol/l
TOOS	:	< 2 mmol/l

Reagent 2 : HDL R2 Reagent

CHE	:	> 2 U/L
COD	:	< 5 KU/L
POD	:	< 10 KU/L

Reagent 3: Direct HDL Calibrator

Concentration see on label

Reagent Preparation

Reagents R1 and R2 are liquid, ready to use. Calibrator reconstitute with 1 ml of deionised water at 20-25°C and mix gently (avoid foaming). Allow to stand for at least 30 minutes until complete reconstitution before use. Store reconstituted calibrator at 2-8°C

Materials Required But Not Provided

- Clean & Dry container.
- Laboratory Glass Pipettes or Micropipettes & Tips
- Colorimeter or Bio-Chemistry Analyzer.

Stability And Storage

The unopened reagents are stable till the expiry date stated on the bottle and kit label when stored at 2–8°C.

Reagents are light-sensitive. Do not let bottles remain open. Keep containers tightly closed.

The reconstituted calibrator is stable for 7 days at 2-8°C.

Specimen Collection And Handling

Use serum or hepairin plasma.

It is recommended to follow NCCLS procedures (or similar standardized conditions).

Stability In Serum / Plasma :

24 hours	:	at 20 – 25°C
7 days	:	at 4 – 8°C
12 weeks	:	at -20°C

Discard contaminated specimens.

Calibration

Calibration with HDL Direct calibrator provided in the kit is recommended.

Quality Control

It's recommended to run normal and abnormal control sera to validate reagent performance.

Normal Value

Adult Male	:	35 - 80 mg/dl
Adult Female	:	42 - 88 mg/dl

It is recommended that each laboratory verify this range or derives reference interval for the population it serves.

Performance Data

Data contained within this section is representative of

performance on Beacon system. Data obtained in your laboratory may differ from these values.

Limit of quantification : 2.32 mg/dl
Linearity : 180 mg/dl
Measuring range : 2.32 – 180 mg/dl

Precision

Intra-assay precision Within run (n=20)	Mean (mg/dl)	SD (mg/dl)	CV (%)
Sample 1	112	3.18	2.84
Sample 2	28	0.85	3.09

Inter-assay precision Run to run (n=20)	Mean (mg/dl)	SD (mg/dl)	CV (%)
Sample 1	31	0.74	2.36

Comparison
A comparison between Liquizyme HDL Cholesterol Direct Reagent kit (y) and a commercially available test (x) using 20 samples gave following results :
y = 1.072 x + 0.705 mg/dl
r = 0.995

Interferences
Following substances do not interfere :
haemoglobin up to 10 g/l, bilirubin up to 40 mg/dl, triglycerides up to 2000 mg/dl. Interference by N-acetylcysteine (NAC), acetoainophen and metamizole causes falsely low results. To carry out the test, blood withdrawal should be performed prior toadministration of drugs.

Warning And Precautions
For *in vitro* diagnostic use. To be handles by entitled and professionally educated person. Reagent of the kit are not classified like dangerous.
Serum used in th manufacture of the calibrator has been tested by FDA-approved methods and found non reactive for hepatitis B surface antigen (HbsAg), antibody to Hepatitis C (HCV), HIV-1 p24 antigen and antibody to HIV1/2. The test procedures do not guarantee that all infectious agents will be detected. Because no test method can offer complete assurance that Hepatitis B virus Hepatitis C virus and HIV ½ or other infectious agents are absent, the material should be handled as potentially infectious.

Waste Management
Please refer to local legal requirements.

Assay Procedure
Primary Wavelength : 578 nm
Secondary Wavelength : 670 nm
Cuvette : 1 cm

Addition Sequence	Reagent Blank	Sample / Calibrator
Reagent 1	450 µl	450 µl
D. D. Water	5 µl	-
Sample / Calibrator	-	5 µl
Mix and incubate 5 min. At 37°C. Then add		
Reagent 2	150 µl	150 µl

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

Calculation

$$\text{HDL-D (mg/dl)} = \frac{\text{Abs. of T}}{\text{Abs. of C}} \times \text{Concentration of Calibrator}$$

Applications for automatic analysers are available on request.

Assay Parameters For Photometers

Mode	End point
Wavelength 1 (nm)	578
Wavelength 2 (nm)	670
Sample Volume (µl)	5
Reagent Volume (µl)	450+150
Incubation time (min.)	5+5
Incubation temp. (°C)	37
Normal Low (mg/dl)	35
Normal High (mg/dl)	80
Linearity Low (mg/dl)	2.32
Linearity High (mg/dl)	180
Blank with	Reagent
Unit	mg/dl

References

1. Dominiczak M, McNamara J. The system of Cardiovascular prevention.103-125; Nauk M, Wiebe D. Warnick G. Measurement of High-Density-Lipoprotein Cholesterol. 221-224. In: Handbook of Lipoprotiein Testing (eds. Rifai, Warnick and Dominiczak) 2nd edition.
2. Barr, D. P., Russ E. M., Eder, H.A., Protein-lipid relationships in human plasma, Am. J. Med., 11;480(1951)
3. Gordon, T. et al., High density lipoprotein as a protective factor against coronary heart disease, Am. J, Med., 62;707 (1977).
4. Castelli, W. P. et al., HDL Cholesterol and other lipids in coronary heart disease, Circulation, 55;767 (1977).
5. National Institutes of Health publication No. 93-3095, September (1993).

Symbols Used On Labels

REF	Catalogue Number		Manufacturer
	See Instruction for Use	LOT	Lot Number
CONT	Content		Storage Temperature
	Expiry Date	IVD	In Vitro Diagnostics

BEA/24/HDL/LS/IFU-02 09/08/2023