BIOGENIX INC. PVT. LTD.

HDL

(Homogenous Direct method)

Document No. BIPL-F-IFU-01, Issue No. 01, Revision No. 00, Effective Date 03/01/2019,

INTENDED USE:

The HDL-Cholesterol reagent is intended for the *in vitro* quantitative determination of High Density Lipoprotein Cholesterol in human serum or plasma. The reagent can assist in the diagnosis and treatment of patients at risk for developing coronary heart disease. Low HDL cholesterol is related to the high risk of coronary heart disease.[1]

INTRODUCTION:

High-density lipoproteins (HDL) compose one of the major classes of plasma lipoproteins. They are synthesized in liver as complexes of apolipoprotein and phospholipid and are capable of picking up cholesterol and carrying 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.[1-8] accurate measurement of HDL-C is of vital importance when assessing patient's risk for CHD.

PRINCIPLE OF THE METHOD:

The HDL-Cholesterol reagent 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.[9] 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 H_2O_2 which is detected through a Trinder reaction.

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

HDL + CHOD + CHER $\longrightarrow$ Fatty Acid + H_2O_2

$2H_2O_2$ + 4-AA + TODB $\xrightarrow{\text{Peroxidase}}$ Quenone + 5 H_2O (λ_{max} = 578nm)

REAGENTS COMPOSITION:

Reagent 1	• MES buffer (pH 6.5): 10 mM • TODB N, N-Bis(4-sulfobutyl)-3- methylaniline:1 mM • Polyvinyl sulfonic acid: 2 mg/L • Polyethylene-glycol-methyl ester: 2 gm/L • $MgCl_2$: 1.6 gm/L • Detergent:0.5 gm/L • EDTA :1.0 gm/L
Reagent 2	• MES buffer (pH 6.5): 10 mM • Cholesterol esterase: 4 U/ml • Cholesterol oxidase: 10 U/ml • Peroxidase: 30U/ml • 4-aminoantipyrine: 2.5 mM • Detergent: 0.5 gm/L

EQUIPMENTS NEEDED BUT NOT PROVIDED

- Any instrument with temperature control of 37 ± 0.5°C that is capable of reading absorbance accurately at 600 nm may be used.
- Controls for validating the performance of the HDL-Cholesterol reagents are sold separately.
- Saline for diluting serum samples and for use as the zero calibrator is not provided.
- Calibrated micropipettes with variable volume, range volume 5-1000 µl;
- Dry thermostat for 37± 0.5 ºC;

REAGENT PREPARATION:

HDL-Cholesterol Assay Reagents (R1, R2) are liquid stable, ready-to-use reagents.

Note: Calibrator sold separately

STORAGE AND STABILITY:

- All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8°C,
- Protected from light and contaminations during their use.
- Do not use reagents :

1. After the expiration date.
2. Signs of reagent deterioration:
3. Presence of particles and turbidity.

COLLECTING AND HANDLING OF SPECIMENS:

Use fresh patient serum and plasma samples (EDTA, Citrate, Li Heparin). Fasting and non-fasting samples can be used. If samples contain HDL cholesterol greater than 184.8 mg/dL, they should be diluted with saline.

PRECAUTIONS:

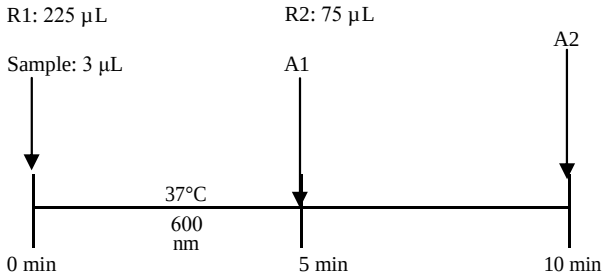
1. For *in vitro* diagnostic use only.
2. Specimens containing human sourced materials should be handled as if potentially infectious using safe laboratory procedures, such as those utlined in Biosafety in Microbiological and Biomedical Laboratories.
3. As with any diagnostic test procedure, results should be interpreted considering all other test results and the clinical status of the patient.
4. Avoid ingestion and contact with skin or mucous membranes. See Material Safety Data Sheet.
5. Reagents are light-sensitive. Do not let bottles remain open. Keep containers tightly closed.

Do not use the reagents after the expiration date labeled on the outer box.

ASSAY PROCEDURES:

Being the HDL all reagents to room temperature before use. Pipette in to test tube and test as fellow**:**

A. Test Scheme for Chemistry Analyzers



Application sheets for use of HDL -Cholesterol Reagents Assay on automated clinical chemistry analyzers are available upon request.

B: Test Scheme for Chemistry Analyzers (Manual Method)

Reagent	**Blank**	**Calibrator**	**Test**
Reagent 1	750 µL	750 µL	750 µL
Calibrator (Conc. see on label)	--	10 µl	--
Specimen	--	--	10 µl
Mix well and incubate for 5 minutes at 37 ˚C in an incubator.			
Reagent 2	250 µL	250 µL	250 µL
Mix well and incubate for 5 minutes at 37 ˚C in an incubator.			

Read the absorbance of Calibrator (C) and test (T) against Blank (B) at 578 nm (546-620 nm).

CALIBRATION:

HDL-cholesterol calibrator should be used to calibrate the BIPL HDL-Cholesterol Reagent. 0.9% Saline should be used as a zero point calibrator. HDL-cholesterol calibrators are provided in lyophilized form and are stable until their expiration date when stored at 2-8°C. Reconstitute contents with distilled water per instructions on vials and mix gently. Let vials equilibrate to room temperature for 30 minutes before use. Reconstituted calibrator is stable for 7 days when capped tightly and stored at 2-8°C.Calibration curve is stable for at least 14 days. Calibration is performed by entering the values as shown on the calibrator bottle labels provided.

HDL Cholesterol calibrator is should be stored at 2-8°C.

Calibration Frequency: Calibration curve is stable for at least 14 days. Calibration is performed by entering the values as shown on the calibrator bottles provided.

SYSTEM PARAMETERS :

Reaction Type (Mode)	:	End Point
Wave Length	:	578 nm (546 – 620)
Flow cell Tem.	:	37˚C
Blank with	:	Reagent
Reagent volume R1	:	750 µl
Reagent volume R2	:	250 µl
Sample Volume	:	10 µl
Linearity	:	180
Calibrator Conc.	:	59.58 mg/dl
Units	:	mg/dl
Low Normal	:	30 mg/dl
High Normal	:	85 mg/dl

QUALITY CONTROL:

It is recommend that each laboratory uses HDL-Cholesterol controls to validate the performance of HDL-Cholesterol reagent. If the results from the controls fall outside the acceptable limits, as determined by their assigned values, the test should not be performed. We recommend that the quality control requirements should be established in accordance with local, state, and/or federal regulations.

RESULTS: SAMPLE CALCULATIONS:

$$\Delta A = A_2 - A_1$$

Conc.of HDL-Cholesterol in serum:=

$$\frac{\Delta A \text{ sample} - \Delta A \text{ blank}}{\Delta A \text{standard} - \Delta A \text{ blank}} \times \text{standard} = \text{mg/dL}$$

UNIT CONVERSION:

HDL-Cholesterol concentration is expressed as mg/dL.

To convert from conventional units to S.I. units, multiply the conventional units by 0.02586.[10]

mg/dL x 0.02586 = mmol/L HDL-Cholesterol mmol/L x 38.66 = mg/dL

Results (in **mg/dL**) are printed out automatically by Hitachi 917. For other instruments, refer to the operator manual for printout instructions.

REFERENCE VALUES:

The HDL activities in human serum samples as follows:

A major risk factor for heart disease	Less than 40 mg/dL
The higher your HDL	40 to 59 mg/dL
The Better	60 mg/dL
Protective against heart disease	60 mg/dL to above

These values are for guidance purpose; each laboratory should establish its own reference range, according to its own geographic area.

LIMITATIONS:

1. A sample with an HDL-Cholesterol level exceeding the linearity limit should be diluted with 0.9% saline and re-assayed incorporating the dilution factor in the calculation of the value.
2. Protect the reagents from direct sunlight.
3. Store the reagents at 2-8°C. Do not freeze the reagents.

PERFORMANCE CHARACTERISTICS:

All performance characteristics were determined using a biochemistry analyzer.

LIMIT OF BLANK

The limit of blank (LOB) of the BIPL HDL-Cholesterol Assay was determined as following: HDL zero calibrator was tested 12.

The LOB = mean + 3SD = 1.06 mg/dL.

ACCURACY

The performance of this assay was compared with the performance of a legally marketed HDL-Cholesterol assay using serum samples. Eighty-four serum samples ranging from 5.7 to 189.3 mg/dL gave a correlation coefficient of 0.987. Linear regression analysis gave the following equation:

This method = 1.048 (reference method) – 4.69 mg/dL

PRECISION:

The precision of the HDL-Cholesterol Reagent was evaluated according to Clinical Laboratory Standards Institute (CLSI) EP5-A guideline. In the study, three serum specimens containing about 30, 55 and 90 mg/dL HDL-Cholesterol were tested on Hitachi 917 with 2 runs per day in duplicates over 20 working days. This method has not been tested or certified by the Cholesterol Reference Method Laboratory Network (CRMLN).

No. of Data N=80	Within Run Precision		
	Level 1: 30 mg/dL HDL	Level 2: 55 mg/dL HDL	Level 3: 90 mg/dL HDL
Mean (µM)	29.00	53.07	90.56
SD (µM)	0.3	0.41	0.84
C_V%	1.0	0.8	0.9

No. of Data N=80	Within Laboratory Precision(ST)		
	Level 1: 30 mg/dL HDL	Level 2: 55 mg/dL HDL	Level 3: 90 mg/dL HDL
Mean (µM)	29.00	53.07	90.56
SD (µM)	0.18	1.36	2.02
C_V%	2.3	2.6	2.2

An additional precision study of the HDL-cholesterol Reagent was conducted in accordance to Clinical and Laboratory Standards Institute (CLSI) EP5-A guideline. In the study, three levels of serum specimens containing about 21, 44, and 160 mg/dL HDL respectively were tested with 2 runs per day in duplicates over 5 working days.

No. of Data N=20	Within Run Precision		
	Level 1: 21 mg/dL HDL	Level 2: 44 mg/dL HDL	Level 3: 160 mg/dL HDL
Mean (µM)	21.63	44.28	159.59
SD (µM)	0.18	0.30	1.77
C_V%	0.90	0.70	1.10

No. of Data N=20	Within Laboratory Precision(ST)		
	Level 1: 21 mg/dL HDL	Level 2: 44 mg/dL HDL	Level 3: 160 mg/dL HDL
Mean (µM)	21.63	44.28	159.59
SD (µM)	0.61	0.79	5.90
C_V%	2.8	1.80	3.7

LINEARITY:

The linearity range of the assay is from 1.06to 180.0 mg/dL in serum. Results below 1.06 mg/dL are invalid. Results that exceed 184.8 mg/dL should be diluted with saline and retested.

INTERFERENCE:

The following substances normally present in serum produced less than 10% deviation at the listed concentrations: Triglycerides: 1000 mg/dL,
Ascorbic acid : 10 mM,
Bilirubin : 40 mg/dL,
Bilirubin conjugates : 30 mg/dL,
Hemoglobin : 1000 mg/Dl

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