ClearChem Diagnostics

HDL

(LIQUID)

3 x 45, 3 x 15 ml

RE – ORDER HDL1210

INTENDED USE

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 desease.[1]

CLINICAL SIGNIFICANCE

High-density lipoproteins (HDL) compose one of the major classes of plasma lipoproteins. They are synthesized in the liver as complexes of apolipoprotein and phospholipids 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. Accurate measurement of HDL-C is of vital importance when assessing patient's risk for CHD.

ASSAY 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 ad selected detergents.[9] LDL, VLDL, and chylomicron (CM) react with PVS and PEGME and the reaction results n 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

HDL + (LDL + VLDL + CM) · PVS/PEGME

HDL + CHOD + CHER ⟶ Fatty Acid + H_2O_2

Peroxidase

$2H_2O_2$ + 4-AA + TODB ⟶ Quenone+ 5 H_2O

(λ_{max}=560nm)

MATERIALS REQUIRED 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 (DZ129A-CON). Saline for diluting serum samples and for use as the zero calibrator is not provided.

REAGENT COMPOSITION

Reagent 1 (R1)	MES buffer (pH 6.5) TODB N, N-Bis (4-sulfobutyl)-3-methylaniline) Polyvinyl sulfonic acid Polyethylene-glycol-methyl ester $MgCl_2$ Detergent EDTA
Reagent 2 (R2)	MES buffer (pH 6.5) Cholesterol esterase Cholesterol oxidase Peroxidase 4-aminoantipyrine Detergent

REAGENT PREPARATION

HDL-Cholesterol Assay Reagent (R1, R2) are liquid stable, ready-to-use reagents. The calibrator is provided in lyophilized form and must be reconstituted with saline before use.

REAGENT STABILITY AND STORAGE

Unopened reagents are stable until the expiration date printed on the outer box when stored at 2-8°C. Reagent on-board stability is at least 60 days. The reagent solutions should be clear. If turbid, the reagents may have deteriorated.

SPECIMEN COLLECTION AND HANDLING

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

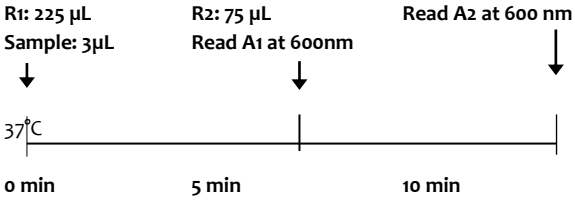
PRECAUTIONS

For in vitro diagnostic use only.

Specimens containing human sourced materials should be handled as if potentially infectious using safe laboratory procedures, such as those outlined in Biosafety in Microbiological and Biomedical Laboratories (HHS Publication Number [CDC] 93-8395). As with any diagnostic test procedure, results should be interpreted considering all other test results and the clinical status of the patient. Avoid ingestion and contact with skin and eyes. See Material Safety Data Sheet. Reagents are light-sensitive. Do not let bottles remain open. Keep container tightly closed. Do not use the reagents after the expiration date labeled on the outer box.

ASSAY PROCEDURES

Test Scheme for Chemistry Analyzers



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

CALIBRATION

HDL-Cholesterol calibrator (DZ129A-Cal) should be used to calibrate the RBI 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 traceable to NIST SRM 195lb and should be stored at 2-8°C.

QUALITY CONTROL

We recommend that each laboratory uses HDL-Cholesterol controls to validate the performance HDL-Cholesterol reagent. A set of low, medium and high HDL-Cholesterol controls is available from RBI (Cat. # DZ129A-Con). If the results from the controls fall outside acceptable limits, as determined by their assigned values, the test should not be performed. We recommend that the quality control testing be established in accordance with local, state, and/or federal regulations.

RESULTS

Sample Calculations

$\Delta A = A_2 - A_1$

Concentration of HDL –Cholesterol in serum:

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

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**.**

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REFERENCE RANGE[10]

The expected values for serum HDL Cholesterol are as follows: Less than 40 mg/dL – A major risk factor for heart disease

40 to 59 mg/dL – The higher your HDL, the better

60 mg/dL and above – An HDL of 60 mg/DL and above is considered protective against heart disease. Each laboratory must establish its own range of expected values.

LIMITATIONS

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. mProtect the reagent from direct sunlight. Store the reagent at 2-8°C. Do not freeze the reagents.

PERFORMANCE CHARACTERISTICS

All performance characteristics were determined at Resolution Biomedical, Inc. using a Hitachi 917 chemistry analyzer.

LIMIT OF BLANK

The limit of blank (LOB) of the RBI HDL-Cholesterol Assay was determined as following: HDL zero calibrator was tested 12 replicates on Hitachi 917. 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 STUDIES

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

Within-Run Precision

	Level 1 30 mg/dL HDL	Level 2 55 mg/dL HDL	Level 3 90 mg/dL HDL
Number of Data Points	80	80	80
Mean (µM)	29.00	53.07	90.56
SD (µM)	0.3	0.41	0.84
CV%	1.0	0.8	0.9

Within-Laboratory Precision (S_r)

	Level 1 30 mg/dL HDL	Level 2 55 mg/dL HDL	Level 3 90 mg/dL HDL
Number of Data Points	80	80	80
Mean (µM)	29.00	53.07	90.56
SD (µM)	0.65	1.36	2.02
CV%	2.3	2.6	2.2

An additional precision study of the Resolution Biomedical, Inc. HDL-Cholesterol Reagent was conducted in accordance to Clinical and Laboratory Standards Institute (CLSI) EPA5-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.

Within-Run Precision

	Level 1 21 mg/dL HDL	Level 2 44 mg/dL HDL	Level 3 160 mg/dL HDL
Number of Data Points	20	20	20
Mean (µM)	21.63	44.28	159.59
SD (µM)	0.18	0.30	1.77
CV%	0.90	0.70	1.10

Within-Laboratory Precision (S_r)

	Level 1 21 mg/dL HDL	Level 2 44 mg/dL HDL	Level 3 160 mg/dL HDL
Number of Data Points	20	20	20
Mean (µM)	21.63	44.28	159.59
SD (µM)	0.61	0.79	5.90
CV%	2.8	1.80	3.7

LINEARITY

The linearity range of the assay is from 1.06 to 184.8 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 at 1000 mg/dL, ascorbic acid at 10 mM, Bilirubin at 40mg/dL, Bilirubin Conjugated at 40 mg/dL, and Hemoglobin at 1000 mg/dL.

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Third Report of the National Cholesterol Education Program (NCEP) Expert Panel of Detection, Evaluation, and Treatment of the High Blood Cholesterol in Adults (Adult Treatment Panel III, or ATP III).

Manufactured for
ClearChem Diagnostics