ClearChem Diagnostics

LDL

(LIQUID)

3 x 45, 3 x 15 ml

RE – ORDER LDL1230

Intended Use

The NBS LDL-Cholesterol Assay is intended for the in vitro quantitative determination of Low Density Lipoprotein Cholesterol in human serum or plasma. The reagents can assist in the diagnosis and treatment of patients at risk of developing coronary heart disease. Elevated LDL cholesterol is the primary target of cholesterol-lowering therapy.

Clinical Significance

Low Density Lipoproteins (LDL) are synthesized in the liver by the action of various lipolytic enzymes on triglyceride-rich Very Low Density Lipoproteins (VLDLs). Specific LDL receptors exist to facilitate the elimination of LDL from plasma by liver parenchymal cells. It has been shown that most of the cholesterol stored in atherosclerotic plaques originates from LDL. For this reason the LDL-Cholesterol concentration is considered to be the most important clinical predictor, of all single parameters, with respect to coronary atherosclerosis. Accurate measurement of LDL-Cholesterol is of vital importance in therapies which focus on lipid reduction to prevent atherosclerosis or reduce its progress and to avoid plaque rupture.

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 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), whereas HDL reacts with the enzymes. Addition of R2 containing a specific detergent releases LDL from the PVS/PEGME complex. The releasedLDL reacts with the enzymes to produce H_2O_2 which is quantified by the Trinder reaction.

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

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

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

$$\text{Detergent}$$
$$\text{(LDL + VLDL + CM)} \cdot \text{PVS/PEGME} \longrightarrow$$

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

$$\text{LDL + CHOD + CHER} \longrightarrow \text{Fatty Acid + } H_2O_2$$

$$2\,H_2O_2 + 4 - AA + \text{TODB} \xrightarrow{\text{Peroxidase}} \text{Quenone + } 5H_2O \quad (\lambda_{MAX}=560\text{nm})$$

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. Application sheets for use of RBI LDL-Cholesterol Reagents on automated clinical chemistry analyzers are available upon request. Controls for validating the performance of the LDL-Cholesterol reagents are sold separately. Saline for diluting serum samples is not provided.

Reagent Composition

Reagent 1 (R1)	MES buffer (pH 6.5) Polyvinylsulfonic acid Polyethyleneglycolmethylester $MgCl_2$ Detergent EDTA 4-aminoantipyrine Cholesterol esterase Cholesterol oxidase Peroxidase
Reagent 2 (R2)	MES buffer (pH 6.5) EDTA Detergent TODB N,N-Bis(4-sulfobutyl)-3-methylaniline)

Reagent Preparation

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

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.

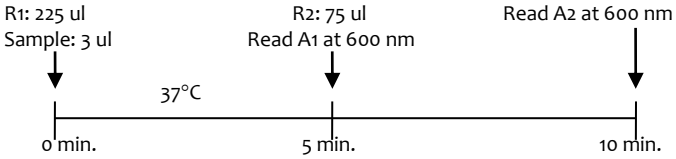
Specimen Collection and Preparation

Use fresh patient serum or plasma samples (EDTA, Citrate). If samples contain LDL cholesterol greater than 250 mg/dL, they should be diluted with saline.

Precautions

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 or mucous membranes. See Material Safety Data Sheet. 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 Procedure



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

Calibration

LDL-cholesterol calibrator is provided and should be used as directed to calibrate the procedure. The catalog number is DZ128A-Cal. LDL-cholesterol calibrator is provided in lyophilized form and is stable until its expiration date when stored at 2-8°C. Reconstitute contents per instructions on vial and mix gently. Let the vials equilibrate to room temperature for 30 minutes before use. Reconstituted calibrators are 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 bottles provided. LDL-Cholesterol calibrator is traceable to NIST SRM 1951b and should be stored at 2–8°C.

Quality Control

We recommend that each laboratory uses LDL-Cholesterol controls to validate the performance of LDL-Cholesterol reagent. A set of low, medium and high LDL-Cholesterol controls is available from RBI Laborat ories (Cat.# DZ128A-Con). 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 your quality control testing follows federal, state, and local guidelines.

Results

LDL-Cholesterol concentration is expressed as mg/dL. To convert from conventional units to S.I. units, multiply the conventional units by 0.02586. mg/dL x 0.02586 = mmol/L LDL-cholesterol mmol/L x 38.66 = mg/dL LDL-cholesterol Results (in **mg/dL**). For instruments, refer to the operator

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manual for printout instructions.

Reference Range

The expected values for serum LDL Cholesterol are as follows:

<100 mg/dL Optimal
100 – 129 mg/dL Near optimal/above optimal
130 – 159 mg/dL Borderline high
160 – 189 mg/dL High
190 mg/dL Very high

Each laboratory must establish its own range of expected values.

Limitations

A sample with a LDL-Cholesterol level exceeding the linearity limit should be diluted with 0.9% saline and reassayed incorporating the dilution factor in the calculation of the value. Anticoagulants containing heparin plasma should not be used. Protect the reagents from direct sunlight. Store the reagents at 2-8°C. Do not freeze the reagents.

Limit of Blank

The limit of blank (LOB) of the RBI LDL-Cholesterol Assay is determined as following: LDL zero calibrator was tested in 12 replicates on Hitachi 917. The LOB = mean+3SD =1.64 mg/dL.

Accuracy

The performance of this assay was compared with the performance of a legally marketed LDL-Cholesterol assay using serum samples. Seventy nine serum samples ranging from 4.0 – 232.0 mg/dL gave a correlation coefficient of 0.9804. Linear regression analysis gave the following equation: y = 1.0883x + 0.6078

Precision

The precision of the RBI LDL-Cholesterol Reagent was evaluated according to Clinical Laboratory Standards Institute (formerly NCCLS) EP5-A guideline. In the study, three serum specimens containing 95, 146 and 210 mg/dL LDL-Cholesterol were tested on Hitachi 917 with 2 runs per day with duplicates over 20 working days. This method has not been certified by the Cholesterol Reference Method Laboratory Network (CRMLN).

Within Run Precision

	Level 1: 95mg/dL LDL	Level 2: 146mg/dL LDL	Level 3: 210mg/dL LDL
N	80	80	80
Mean	97.14	147.37	211.47
SD	1.00	1.19	1.38
CV%	1.0%	0.8%	0.7%

Within-Laboratory Precision

	Level 1: 95mg/dL LDL	Level 2: 146mg/dL LDL	Level 3: 210mg/dL LDL
N	80	80	80
Mean	97.14	147.37	211.47
SD	1.55	2.23	2.98
CV%	1.6%	1.5%	1.4%

Additional precision study of the RBI LDL-cholesterol Reagent was conducted according to modified Clinical and Laboratory Standards Institute (CLSI) EP5-A guideline. In the study, one level of serum specimen containing about 70 mg/dL LDL were tested with 2 runs per day in duplicates over 5 working days.

Within Run Precision

	Level 1: 70 mg/dL LDL
N	20
Mean	71.19
SD	0.50
CV%	0.70%

Within-Laboratory Precision

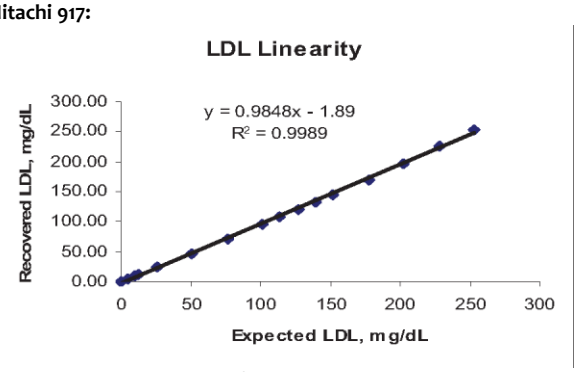
	Level 1: 70 mg/dL LDL
N	20
Mean	71.19
SD	1.15
CV%	1.60%

Linearity

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).

Sixteen levels of linearity set were prepared by diluting a serum sample containing 250 mg/dL of LDL with saline according to Clinical and Laboratory Standards Institute (formerly NCCLS) EP6-A. The LDL recovered is plotted against the expected on the

Hitachi 917:



The linearity is up to 250 mg/dL.

Interference

Interference for the RBI LDL-Cholesterol Assay was evaluated on Hitachi 917. The following substances normally present in serum produced less than 10% deviation at the listed concentrations: Triglyceride at 1000 mg/dL, Ascorbic Acid at 10 mM, Bilirubin at 40 mg/dL, Bilirubin Conjugate at 30 mg/dL, Hemoglobin at 1000 mg/dL.

References

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