LDL-c Direct (Homogeneous.)

INTENDED USE

Bioline LDL is used for the direct quantitative determination of low density lipoprotein cholesterol (LDL-C) in human serum or plasma on automated analyzer For *in vitro* diagnostic use only.

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.

METHOD AND PRINCIPLE

The Direct LDL Cholesterol Reagent is a two-part, liquid stable method for directly measuring LDL-C levels in serum or plasma. The method depends on the properties of a unique detergent which eliminates the need for any off-line pre-treatment or centrifugation steps. This detergent (Reagent 1) solubilizes only the non-LDL lipoprotein particles. The cholesterol released is consumed by cholesterol esterase and cholesterol oxidase in a non-color forming reaction. A second detergent (Reagent 2) solubilizes the remaining LDL particles and a chromogenic coupler allows for color formation. The enzyme reaction with LDL-C in the presence of the coupler produces color that is proportional to the amount of LDL cholesterol present in the sample.

REAGENT COMPOSITION

1. Direct LDL Cholesterol Reagent 1:
   Buffer 100 mmol/L (pH 7.0), Cholesterol esterase from Pseudomonas, 800 U/L; Cholesterol oxidase form Nocardia sp, 500 U/L; Peroxidase from Horseradish 800 U/L; 4-amino antipyrine, 1 mmol/L; Preservative..
2. Direct LDL Cholesterol Reagent 2:
   Buffer 100 mmol/L (pH 7.0), 2: N,N-bis (4-sulfhobutyl)- m-Toluidine- disodium (DSBmT) 1.2%; Preservative.

WARNINGS AND PRECAUTIONS

1. Reagent is intended for *in vitro* diagnostic use only.
2. Do not pipette by mouth
3. All specimens used in this test should be considered potentially infectious. Universal precautions as they apply to your facility should be used for handling and disposal of materials during and after testing.
4. Do not use the reagents beyond the expiration date printed on the kit label.

STORAGE AND STABILITY

All reagents are stable until the expiration date on the label when stored at 2 to 8°C.

REAGENT DETERIORATION

The reagent should be clear. Cloudiness would indicate deterioration.

Do not use the product if there is visible evidence of biological, chemical or physical deterioration.

SPECIMEN COLLECTION AND STORAGE

Serum, EDTA-treated or heparinized plasma are the recommended specimens. Patients are not required to fast prior to blood collection.

Serum : Collect whole blood by venipuncture and allow to clot. Centrifuge and remove the serum as soon as possible after collection (within 3 hours).

Plasma : Specimens may be collected in EDTA or heparin. Centrifuge and remove the plasma as soon as possible after collection (within 3 hours).

If not analyzed promptly, specimens may be stored at 2-8°C for up to 5 days. If specimens must be stored for more than 5 days, they may be frozen at –80°C.

INTERFERENCES

All interference studies were conducted according to the procedures recommended in NCCLS guideline No. EP7-P for interference testing in clinical chemistry. Hemoglobin at levels up to 400 mg/dl, Bilirubin at levels up to 20 mg/dl and Triglycerides to 1500 mg/dl were found to exhibit negligible interference (<5%) on this method. Samples with levels of interfering substances higher than the upper limits should be diluted with physiological saline before assaying. Multiply the result obtained from the manual dilution by the appropriate dilution factor. For a comprehensive review of drug interference on serum LDL cholesterol levels see Young et al.

ASSAY PROCEDURE FOR SEMIAUTO ANALZYER.

Wavelength : (578-600) nm

Temperature : 37 °C

	Blank	Calibrator	Sample
Reagent R1	750 µL	750 µL	750 µL
Calibrator	-	10 µL	-
Sample	-	-	10 µL

Mix & incubate at 37 °C for 5 minutes, then add

Reagent R2	250 µL	250 µL	250 µL

Mix and read the optical density of calibrator and sample after 5 min of incubation at 600-630 nm.

CALCULATIONS

Concn of patient (mg/dl) = $\frac{\text{Abs.(patient)}}{\text{Abs.(Calibrator)}}$ X Concentration of Calibrator (mg/dl)

Example : Abs.(patient)=0.32

Abs.(standard)=0.28

Concentration of Calibrator=114 mg/dl

Concentration of patient (mg/dl) = $\frac{0.32}{0.28}$ x 114 =130.2 mg/dl

CALIBRATION

The Direct LDL Cholesterol Calibrator is required for calibration. Calibrate with each bottle change or lot change or if control results are found to be out of range. The values of the calibrator were assigned by procedures traceable to the National Reference System for Cholesterol (NRS/CHOL). Refer to Direct HDL/LDL Cholesterol Calibrator package insert for

instructions. If control results are found to be out of range, the procedure should be re-calibrated.

LIMITATIONS

1. Anticoagulants containing citrate should not be used.
2. Protect the reagents from direct sunlight.
3. Samples with values greater than 400 mg/dl must be diluted 1:1 with saline and re-assayed. Multiply the result by two.

QUALITY CONTROL

Reliability of test results should be routinely monitored with control materials that reasonably emulate the performance of patient specimens.[10] Quality control materials are intended for use only as monitors of accuracy and precision. Any HDL/LDL controls would be suitable for use with this assay. The recovery of control values within the appropriate range should be the criteria used in evaluation of future assay performance. Controls should be run with every working shift in which LDL-C assays are performed. It is recommended that each laboratory establish its own frequency of control determination. Quality control requirements should be determined in conformance with local, state, and/or Federal regulations or accreditation requirements.

EXPECTED VALUES

The following NCEP recommendations for patient classifications are suggested for the prevention and management of coronary heart disease:

LDL Cholesterol	**Classifications**
<100mg/dl	Optimal
100-129mg/dl	Near Optimal/Above Optimal
130-159 mg/dl	Border line High Risk
160-189mg/dl	High Risk
≥190 mg/dl	Very High Risk

It is highly recommended that each laboratory establish its own range ofexpected values.

PERFORMANCE CHARACTERISTICS

Assay Range: 5-450 mg/dl

Accuracy: Accuracy of the Direct LDL Cholesterol Reagent method was verified by comparison to the reference method (Auto LDL Cholesterol) on Hitachi 717. There were 52 serum samples in this study.

General Technical Parameters

Mode	**End Point**
Wavelength (Filter)	**(578 -600) nm**
Reaction Direction	**Increasing**
Reagent Blank	**Yes**
Sample Vol.	**10 µL**
Reagent Vol.	**1000 µL**
Incubation Time	**5+5min**
Reagent Blank Abs (Max.)	**NMT 0.100 Abs**
Calibration Method	**1 - Point**
Calibrator (Conc.)	**Refer Calibrator vial**
Linearity	**450 mg/dL**
Decimal Places	**1**
Temp.	**37 °C**
Unit	**mg/dL**

REFERENCES

1. Gotto, A.M., Lipoprotein Metabolism and the etiology of Hyperlipidemia, Hospital practice, 23:Suppl. 1,4 (1988).
2. Crouse, J.R., et al., Studies of Low Density Lipoprotein Molecular Weight in Human Beings with Coronary Artery Disease, J. Lipid Res., 26:566 (1985).
3. Badimon, J.J., Badimon L., Fuester V., Regression of Athroscierotic Lesions by High-density lipoprotein Plasma fraction in the Cholesterol-Fed Rabbit, Journal of Clinical Investigation, 85:1234-41 (1990).
4. Castelli, W.P., et al., Cholesterol and other Lipids in coronary heart disease, Circulation, 55-767 (1977).