

# LDL Cholesterol (Direct)

## 1.0 INTENDED USE

For the direct quantitative determination of low-density lipoprotein cholesterol (LDL-C) in human serum or plasma. For In Vitro diagnostic use only.

## 2.0 BACKGROUND

### 2.1 METHOD AND HISTORY

Plasma lipoproteins are spherical particles containing varying amounts of cholesterol, triglycerides, phospholipids and proteins. The phospholipid, free cholesterol and protein constitute the outer surface of the lipoprotein particle, the inner core contains mostly esterified cholesterol and triglycerides. These particles serve to solubilize and transport cholesterol and triglycerides in the bloodstream.

The relative proportions of protein and lipid determine the density of these plasma lipoproteins and provide a basis on which to begin their classification (11.1). These classes are: very low-density lipoproteins (VLDL), low-density lipoproteins (LDL) and high-density lipoprotein (HDL). Numerous clinical studies have shown that the different lipoprotein classes have varied effects. (11.2-11.4). The studies all point to LDL cholesterol as the key factor in the pathogenesis of atherosclerosis and coronary artery disease (CAD) (11.2-11.8), while HDL cholesterol has often been observed to have a protective effect. Even within the normal range of total cholesterol concentrations, an increase in LDL cholesterol can occur with an associated increased risk for CAD (11.4).

Over the years a variety of methods have been employed for the determination, or estimation, of LDL cholesterol. The Friedewald equation, in a variety of forms, has been most frequently used for the estimation of LDL cholesterol. However, its usefulness is limited and its accuracy has been questioned. Determination of LDL cholesterol by beta-quantification is recognized as the reference method but the procedure is so cumbersome relatively few laboratories use this method. A recent method using immunoseparation has become popular. However, this method still requires sample pretreatment prior to cholesterol determination, making it unsuitable for full automation of the procedure. The method presented here offers direct determination of LDL cholesterol in a two part, liquid stable reagent that is easily adapted to most automated chemistry analyzers.

### 2.2 TEST PRINCIPLE

The 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 pretreatment 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.

HDL, VLDL Chylomicrons	Solubilized HDL VLDL, Chylomicrons	Consumed HDL, VLDL, Chylomicrons (No color development)
---------------------------	---------------------------------------	--

Detergent/Reagent 1	Detergent/Reagent 2	
LDL	Non-solubilized LDL Cholesterol	Solubilized LDL Cholesterol
Solubilized LDL Cholesterol	+ H2O2	
	Cholesterol Esterase Cholesterol Oxidase	
H2O2 + DSBmT + 4-AA		Color Development (Measured Biochromatical at 546 & 660nm)
	Peroxidase	

## 3.0 SPECIMEN COLLECTION AND STORAGE

### 3.1 SPECIMEN COLLECTION

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) (11.10).

Plasma: Specimens may be collected in EDTA or heparin. Centrifuge and remove the plasma as soon as possible after collection (within 3 hours) (11.10).

### 3.2 SPECIMEN STORAGE

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

### 3.3 INTERFERENCES

All interference studies were conducted according to the procedures recommended in NCCLS guideline No. EP7-P for interference testing in clinical chemistry (11.12). Hemoglobin at levels up to 400 mg/dl and Bilirubin at levels up to 20 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 (11.13)

## 4.0 MATERIALS (1 X 30 ml, 1 X 10 ml)

(1 X 60 ml, 1 X 20 ml)

The reagents necessary for the determination of LDL Cholesterol are included in the kit.

### 4.1 REAGENT COMPOSITION

Components	Appearance	Ingredients
Reagent 1	Liquid	MES Buffer (pH 6.3)

		Detergent 1
		Cholesterol esterase
		Cholesterol oxidase
		Peroxidase
		4-aminoantipyrine
		Ascorbic acid oxidase
		Preservative
Reagent 2	Liquid	MES Buffer (pH 6.3)
		Detergent 2
		N,N-bis(4-sulfobutyl)-m-
		Toluidine-disodium (DSBmT)
		Preservative

Cholesterol Oxidase from Nocardia sp., Cholesterol Esterase from Pseudomonas sp., Peroxidase from Horseradish, Ascorbic Acid Oxidase from Cucurbita sp.

**4.2 WARNINGS AND PRECAUTIONS**

- 4.2.1 For In Vitro diagnostic use.
- 4.2.2 Do not pipette by mouth.
- 4.2.3 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.
- 4.2.4 Do not use the reagent after the expiration date printed on the kit.

**4.3 WORKING REAGENT PREPARATION**

Reagent 1: Reagent 1 is ready to use as packaged.

Reagent 2: Reagent 2 is ready to use as packaged..

**4.4 REAGENT STORAGE AND STABILITY**

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

**4.5 ADDITIONAL MATERIALS REQUIRED**

- 4.5.1 LDL Cholesterol (Direct) Calibrator..
- 4.5.2 Lipid Control..
- 4.5.3 Automated clinical chemistry analyzer capable of accommodating two-reagent assays.

**5.0 PROCEDURE**

Below is a general example of the LDL test procedure for an automated analyzer. All analyzer applications should be validated in accordance with NCEP and CLIA recommendations (11.10). For assistance with applications on automated analyzers, please contact Biotron Diagnostics technical service department at 1-800-595 8766.

		37°C		37°C	
Sample + Reagent 1			Reagent 2		Measurement
3µl	300µl	5 min	100µl	5 min	of Absorbance 2
					(660/546nm)
		Measurement of			
		Absorbance 1			
		(660/546nm)		LDL-C Result	

**6.0 LIMITATIONS**

- 6.0.1 Anticoagulants containing citrate should not be used.
- 6.0.2 Protect the reagents from direct sunlight.
- 6.0.3 Samples with values greater than 700mg/dl must be diluted with 1:1 with saline and reassayed. Multiply the results by two.

## 7.0 CALIBRATION

The LDL Cholesterol Calibrator is required for calibration. The value of the LDL Calibrator was assigned by procedures traceable to the National Reference System for Cholesterol (NRS/CHOL). Refer to LDL Cholesterol Calibrator package insert for instructions. If control results are found to be out of range the procedure should be recalibrated.

## 8.0 QUALITY CONTROL

Reliability of test results should be routinely monitored with control materials that reasonably emulate performance on patient specimens (11.10). Quality control materials are intended for use only as monitors of accuracy and precision. 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 their own frequency of control determination. Quality control requirements should be performed in conformance with local, state, and/or Federal regulations or accreditation requirements.

## 9.0 RESULTS

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

Example: mg/dl X 0.02586=mmol/L LDL-C

### 9.1 EXPECTED VALUES

The following NCEP recommendations for patient classifications are suggested for the prevention and management of coronary heart disease(11.8).

<u>LDL Cholesterol</u>	<u>Classification</u>
<130mg/dl (3.36mmol/L)	Desirable
130-159mg/dl (3.36-4.11mmol/L)	Borderline High Risk
≥160mg/dl (4.14mmol/L)	High Risk

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

## 10.0 SPECIFIC PERFORMANCE CHARACTERISTICS

10.1 ASSAY RANGE: 0-700 mg/dl.

10.2 ACCURACY

Accuracy of the LDL Cholesterol Reagent method was verified by comparison to the reference method (β-quantification followed by cholesterol analysis),(11.10) another automatable LDL cholesterol method, and the LDL immunoseparation method. Studies comparing the LDL Cholesterol method to the reference method produced the following results:

<u>Method</u>	<u>LDL Cholesterol</u>	<u>Reference Method</u>
n	40	40
Mean LDL Cholesterol (mg/dl)	130.7	136.7
Range (mg/dl)	18-231	24-251
Standard Deviation (mg/dl)	43.8	45.9
Regression Analysis	y=0.92x + 5.22mg/dl	
Correlation Coefficient	r=0.962	

Studies comparing LDL Cholesterol method to the other automatable LDL Cholesterol method produced the following results:

<u>Method</u>	<u>LDL Cholesterol</u>	<u>Automatable Method</u>
n	45	45
Mean LDL Cholesterol (mg/dl)	106.2	113.6
Range (mg/dl)	15-186	19-192
Standard Deviation (mg/dl)	33.9	36.0
Regression Analysis	y=0.93x + 0.41mg/dl	
Correlation Coefficient	r=0.988	

Studies comparing LDL Cholesterol method to the LDL Cholesterol immunoseparation method produced the following results:

<u>Method</u>	<u>LDL Cholesterol</u>	<u>Immunoseparation Method</u>
n	31	31
Mean LDL Cholesterol (mg/dl)	117.9	120.4
Range (mg/dl)	50-219	39-231
Standard Deviation (mg/dl)	40.0	42.0
Regression Analysis	y=0.90x + 9.61mg/dl	
Correlation Coefficient	r=0.944	

### 10.3 PRECISION

Within-day precision for the direct HDL Cholesterol Reagent was determined following a modification of NCCLS document EP5-T2(11.17). Within-Day precision studies produced the following results:

<u>Sample</u>	<u>LOW</u>	<u>MID</u>	<u>HIGH</u>
n	20	20	20
Mean HDL Cholesterol (mg/dl)	37	122	187
Standard Deviation (mg/dl)	1.5	4.2	6.3
Coefficient of Variation (%)	4.1	3.4	3.4

Day- to-Day precision was also determined following a modification of NCCLS document EP5-T2(11.17) Day-to-Day precision studies produced the following results.

Sample	LOW	MID	HIGH
n	20	20	20
Mean HDL Cholesterol (mg/dl)	38	135	222
Standard Deviation (mg/dl)	2.1	7.9	7.5
Coefficient of Variation (%)	5.4	5.9	3.4

#### 10.4 SENSITIVITY

The analytical sensitivity for LDL Cholesterol was determined to be 0.0013 absorbance units per 1mg/dl of LDL cholesterol.

#### 11.0 REFERENCES

- 11.1 Gotto A.M., Lipoprotein metabolism and the etiology of hyperlipidemia, Hospital practice, 23:Suppl. 1,4 (1988).
- 11.2 Crouse J.R. et al., Studies of low density lipoprotein molecular weight in human beings with coronary artery disease, J. Lipid Res., 25:566(1985).
- 11.3 Badimon J.J., Badimon L., Fuester V., Regression of Atherosclerotic Lesions by High-Density Lipoprotein Plasma Fraction in the Cholesterol-Fed Rabbit, Journal of Clinical Investigation, 1990; 85:1234-41.
- 11.4 Castelli W.P. et al., Cholesterol and other lipids in coronary heart disease, Circulation, 55:767 (1977).
- 11.5 Barr D.P., Russ E.M., Eder H.A., Protein-lipid relationships in human plasma, Am. J. Med., 11:480 (1951).
- 11.6 Gordon T. et al., High density lipoprotein as a protective factor against coronary heart disease, Am. J. Med., 62:707 (1977).
- 11.7 Williams P., Robinson D., Baily A., High density lipoprotein and coronary risk factor, Lancet, 1:72 (1979).
- 11.8 Kannel W.B., Castelli W.P., Gordon T., Cholesterol in the prediction of atherosclerotic disease; New perspectives based on the Framingham study, Am. Intern. Med., 90:85 (1979).
- 11.9 National Institutes on health publication No. 93-3095, September 1993.
- 11.10 Warnick G. Russell, Wood Peter D., National Cholesterol Education Program Recommendations for Measurement of High-Density Lipoprotein Cholesterol; Executive Summary, Clinical Chemistry, Vol. 41, No. 10, 1995.
- 11.11 Grundy S.M. et al., Summary of the Second Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel II), JAMA 1993, 269:23;3015-3023.
- 11.12 National Committee for Clinical Laboratory Standards, National Evaluation Protocols for Interference Testing, Evaluation Protocol Number 7, vol. 4, No. 8, June 1984.
- 11.13 Young, D.S., Effects of Drugs on Clinical Laboratory Tests, 3rd ed., AACC Press, Washington DC, 1990, 3-104 thru 3-106.
- 11.14 Tietz N.W., Clinical Guide to Laboratory Tests, W.B. Saunders Co., Philadelphia, 1986, p. 256.
- 11.15 Carey RN , Garber CC. Evaluation of Methods. In Kaplan LA, Pesce AJ, eds. Clinical Chemistry: theory, analysis and correlation. Third Edition.st.Louis: The CV Mosby Company.
- 11.16 Westgard JO, Carey RN. Wold S. Criteria for judging precision and accuracy in method development and evaluation. Cliical Chemistry 1974;20:825-833.
- 11.17 NCCLS document Evaluation of Precision Performance of Clinical Chemistry Devices 2nd Ed. 1992.