POINTE SCIENTIFIC, INC.
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autoLDL™ Cholesterol Reagent Set

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.

Summary
Plasma lipoproteins are spherical particles that contain 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 for their classification.[1] The 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.[2-4] The studies all point to LDL cholesterol as the key factor in the pathogenesis of artherosclerosis and coronary artery disease (CAD),[2-8] 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 risk for CAD.[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 is still requires sample pre-treatment 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.

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
Components	Appearance	Ingredients
Reagent 2	Liquid	MES Buffer (pH 6.3)
		Detergent 2 N,N-bis (4-sulfhobutyl)-m-Toluidine-disodium (DSBmT) Preservative

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

Principle
The autoLDL™ 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.

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

LDL ---------- Detergent/Rgt1 ----------➢ Non-solubilized LDL Cholesterol ---------- Detergent/Rgt2 ----------➢ Solubilized LDL Cholesterol

Solubilized LDL Cholesterol ---------- Cholesterol Esterase, Cholesterol Oxidase ----------➢ + H_2O_2

H_2O_2 + DSBmT + 4-AA ---------- Peroxidase ----------➢ Color Development (Measured Bichromatically at 546 & 660nm)

Reagent Preparation
Reagent 1: Reagent 1 is ready to use.
Reagent 2: Reagent 2 is ready to use.

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

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.

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).[10]

Plasma: Specimens may be collected in EDTA or heparin. Centrifuge and remove the plasma as soon as possible after collection (within 3 hours).[10]

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.[12] 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.[13]

Materials Provided

autoLDL™ Cholesterol Reagent Set			
Catalog No.	L7574-40	L7574-80	L7574-320
Reagent 1	30mL	60mL	240mL
Reagent 2	10mL	20mL	80mL

Materials Required but not Provided

1. Pointe Scientific autoHDL/LDL™ Calibrator, Cat. No. H7545-CAL.
2. LDL cholesterol controls.
3. Automated clinical chemistry analyzer capable of accommodating two-reagent assays.

Procedure

Below is general example of the autoLDL™ test procedure for an automated analyzer. All analyzer applications should be validated in accordance with NCEP and CLIA recommendations.[10] For assistance with applications on automated analyzers, please contact Pointe Scientific's Technical Service Department at (800)445-9853.

		37°C		37°C		
Sample +	Reagent 1	----➢	Reagent 2	----➢	Measurement (Absorb. Difference
3ul	300ul	5min.	100ul	5min.	between 660nm & 546nm)

I
I
I
V
LDL-C Result

Limitations

1. Anticoagulants containing citrate should not be used.
2. Protect the reagents from direct sunlight.
3. Samples with values greater than 700 mg/dl must be diluted 1:1 with saline and re-assayed. Multiply the result by two.

Calibration

The autoHDL/LDL™ Cholesterol Calibrator is required for calibration. The values of the calibrator were assigned by procedures traceable to the National Reference System for Cholesterol (NRS/CHOL). Refer to autoHDL/LDL™ Cholesterol Calibrator package insert for instructions. If control results are found to be out of range, the procedure should be re-calibrated.

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. 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 determined in conformance with local, state, and/or Federal regulations or accreditation requirements.

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

Expected Values

The following NCEP recommendations for patient classifications are suggested for the prevention and management of coronary heart disease:[8]

LDL Cholesterol	Classifications
<130mg/dl (3.36mmol/L)	Desirable
130-159m/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.

Specific Performance Characteristics

Assay Range: 0-700 mg/dl

Accuracy: Accuracy of the autoLDL™ Cholesterol Reagent method was verified by comparison to the reference method (*B*-quantification followed by cholesterol analysis),[10] another automatable LDL cholesterol method, and the Direct LDL immunoseparation method. Studies comparing the autoLDL™ Cholesterol method to the reference method produced the following results:

Method	autoLDL™ Cholesterol	Reference Method
N	40	40
Mean LDL Cholesterol	130.7	136.7
Range (mg/dl)	18-231	24-251
Standard Deviation (mg/dl)	43.8	45.9
Regression Analysis	Y=0.92x + 5.22 mg/dl	
Correlation Correlation	R=0.962	

Studies comparing the autoLDL™ Cholesterol method to another automatable LDL cholesterol method produced the following results:

Method	autoLDL™ Cholesterol	Automatable Method
N	45	45
Mean LDL Cholesterol	106.2	113.6
Range (mg/dl)	15-186	19-192
Standard Deviation (mg/dl)	33.9	36.0
Regression Analysis	Y=0.93x – 0.41 mg/dl	
Correlation Coefficient	R=0.988	

Studies comparing the autoLDL™ Cholesterol method to the Direct LDL immunoseparation method produced the following result:

Method	autoLDL™ Cholesterol	Immunoseparation Method
N	31	31
Mean LDL Cholesterol	117.9	120.4
Range (mg/dl)	50-219	39-231
Standard Deviation (mg/dl)	40.0	42.0
Regression Analysis	Y=0.90x – 9.61 mg/dl	
Correlation Coefficient	R=0.944	

Precision:

Within-Day precision for the autoLDL™ Cholesterol Reagent was determined following a modification of NCCLS document EP5-T2.[17] Within-Day precision studies produced the following results:

Sample	LOW	MID	HIGH
N	20	20	20
Mean LDL 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.[17] Day-to-Day precision studies produced the following results:

Sample	LOW	MID	HIGH
N	20	20	20
Mean LDL 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

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

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).
5. Barr, D.P., Russ, E.M, Elder, H.A., Protein-Lipid Relationships in Human Plasma, Am. J. Med. 11:480 (1951).
6. Gordon, T., et al, High Density Lipoprotein as a Protective Factor Against Coronary Heart Disease, Am. J. Med., 62:707 (1977).
7. William, P., Robinson, D., Baily A., High Density Lipoprotein and Coronary Risk Factor, Lancet, 1:72 (1979).
8. Kannel, W.B., Castelli W.P., Gordon, T., Cholesterol in the Prediction of Artheriosclerotic Disease; New Perspectives Based on the Framingham Study, Am. Intern. Med., 90:85 (1979).
9. National Institutes on Health Publication no. 93-3095, September 1993.
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. 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.
12. National Committee for Clinical Laboratory Standards, National Evaluation Protocols for Interference Testing, Evaluation Protocol Number 7, Vol. 4, No. 8, June 1984.
13. Young, D.S. Effects of Drugs on clinical Laboratory Tests, 3rd ed., AACC Press, Washington, D.C., 1990, 3-104 thru 3-106.
14. Tietz, N.W., Clinical Guide to Laboratory Tests, W.B. Saunders Co., Philadelphia, 1986, p. 256.
15. Carey, R.., Gerber, C.C., Evaluation of Methods. In Kaplan LA, Pesce, A.J., eds. Clinical Chemistry: theory, analysis and correlation. Third Edition. St. Louis: The CV Mosby Company.
16. Westgard, J.O., Carey, R.N., Wold, S., Criteria for judging precision and accuracy in method development and evaluation. Clinical Chemistry 1974:20:825-833.
17. NCCLS document "Evaluation of Precision Performance of Clinical Chemistry Devices" 2nd Ed. 1992.

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