Direct LDL-Cholesterol LiquiColor®
Procedure N° BQ037

For the Quantitative Determination of Low Density Lipoprotein (LDL) Cholesterol in Human Serum or Plasma.

Summary and Principle

Lipoproteins are spherical-shaped particles that contain varying amounts of cholesterol, triglycerides, phospholipids and proteins. The phospholipids and proteins make up the outer surface of the lipoprotein particle, while the core consists mostly of cholesterol in esterified form and triglycerides. The purpose of the lipoprotein particles is to transport cholesterol and triglyceride through the bloodstream. The relative amounts of the protein and lipid constituents determine the density of the lipoprotein particles and provide a basis for their classification[1]. These classes are: very-low-density lipoproteins (VLDL), low-density lipoproteins (LDL) and high-density lipoproteins HDL). There have been many clinical studies that have shown that these lipoprotein particles have very distinct and varied effects on the risk of coronary heart disease (CHD)[2]. High LDL-C levels have repeatedly been associated with an increased risk of coronary heart disease and coronary artery disease[3-8]. Thus, the determination of serum LDL cholesterol has been recognized as a useful tool in identifying high-risk patients. Historically, most laboratories have used the Friedwald equation to calculate the LDL cholesterol based on results from 3 separate assays: Total cholesterol, HDL cholesterol and triglycerides. This equation has many limitations including the fact that the triglycerides value cannot exceed 400 mg/dL in the sample As a result, the routine determination of LDL-C has suffered from both long turnaround times and poor reproducibility.
The Direct LDL Cholesterol LiquiColor® is a homogenous method for directly measuring serum LDL-C levels without the need for any off-line pretreatment or centrifugation steps. The method employs a two-reagent system. The first reagent (R1) contains a combination of detergent, organic and inorganic phosphoric acid compounds which specifically binds HDL, VLDL and chylomicrons leaving the LDL particles exposed. The second reagent (R2) contains enzymes which then reacts with the LDL cholesterol present in the sample. Consequently, only the LDL cholesterol is subject to cholesterol measurement.

Reagents

Direct LDL LiquiColor® Buffer (R1), Cat No. BQ037a

Magnesium Sulfate	2.5	mmol/L
HDAOS	0.8	

Direct LDL LiquiColor® Enzyme (R2), Cat No. BQ037b

Cholesterol Oxidase	> 5,000	U/L
Cholesterol Esterase	> 800	U/L
Peroxidase	> 15 000	U/L
4-aminoantipyrine	0.5	mmol/L

Good's Buffer (pH 6.8 ± 0.1)
Surfactants and preservative.

Precautions: *For In Vitro Diagnostic Use Only.* Do not pipette by mouth. 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. Do not use reagents after the expiration date printed on their respective labeling.
NOTE: This reagent was not tested or certified by the CRMLN (Cholesterol Reference Method Laboratory Network).

Reagent Preparation: Reagents are supplied ready to use.

Reagent Storage and Stability: Reagents are stable stored at 2-8°C until expiration date on their respective labeling. Once opened, contamination must be avoided.

Materials Required But Not Provided

Direct HDL/LDL-Cholesterol Calibrator, Cat. No. AX1580 Automated Chemistry Analyzer capable of utilizing a two-reagent system

Specimen Collection and Preparation

Blood should be collected following a 12-hour fast. Specimen may be serum, or plasma collected with sodium or lithium heparin as anticoagulants, do not use EDTA. Avoid hemolysis.
Serum - Collect whole blood by venipuncture and allow to clot. Centrifuge and remove the serum as soon as possible after collection (within 3 hours)[9]. **Plasma -** Use Li-heparin or Na-heparin plasma to eliminate the possibility of a change in lipoprotein composition due to the time necessary for coagulation. Centrifuge and remove the plasma as soon as possible after collection (within 3 hours)[9]. EDTA plasma causes decreased results.
Sample Stability: If not analyzed promptly, specimens may be stored at 2-8°C for up to 1 week. If specimens need to be stored for more than 1 week, they may be preserved at less than -20°C for up to 1 month. For storage periods of 1 month to 2 years, samples should be preserved at -70°C[9].
Interfering Substances: Anticoagulants containing EDTA should not be used. The test is not influenced by hemoglobin values up to 500 mg/dL, bilirubin levels up to 40 mg/dL, ascorbic acid up to 50 mg/dL, and chylomicrons up to 3000 mg/dL. Refer to the work of Young for a review of drug effects on serum LDL cholesterol levels[10].

Automated Analyzers

Below is a general example of the Direct LDL Cholesterol LiquiColor® test procedure for an automated analyzer. All analyzer applications should be validated in accordance with NCEP and CLIA recommendations[11].

Sample + Reagent 1		Incub. 37°C	+ Reagent 2	Incub. 37°C	Measure absorbance difference between 700 nm & 600 nm)
3 µL	300 µL	5 min.	100 µL	5 min.	OD_{700}-OD_{600}=> LDL-C result

Quality Control: Ser-T-Fy® I, Normal Control Serum, Cat. No. FT7670 and Ser-T-Fy® II, Abnormal Control Serum, Cat. No. FT7680 are recommended for each run. Other commercially available controls with direct LDL values assayed by this method are also suitable. Direct LDL determined in these materials, by this procedure should fall within the ranges stated for the controls. Two levels of controls should be analyzed with each run.

Calibration: The use of the Direct HDL/LDL Calibrator (available separately) is required for calibration of this assay. Refer to the Direct HDL/LDL Calibrator package insert for instructions. If control results are found to be out of range, the procedure should be recalibrated. The instrument manufacturer's calibration guidelines should be followed to calibrate your analyzer.

Limitations

Anticoagulants containing EDTA should not be used. Samples with values greater than 520 mg/dL must be diluted 1:1 with saline and re-assayed. Multiply the result by two.

Results

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

Expected Values

The following NCEP recommendations for patients classifications are suggested for the prevention and management of coronary heart disease[11].

Desirable: < 100 mg/dL (< 2.58 mmol/L)
Borderline High Risk: 130 - 159 mg/dL (3.36 - 4.11 mmol/L) High Risk: 160 -189 mg/dL (4.14 -4.88 mmol/L)
Very High Risk: > 190 mg/dL (> 4.90 mmol/L)

It is recommended that each laboratory establish its own range of expected values, since differences exist between instruments, laboratories, and local populations.

Performance Characteristics[12]

Data was derived on Hitachi® 917 analyzer.

Accuracy: Linear regression analysis of 62 serum samples with LDL cholesterol levels ranging from 22 to 178 mg/dL was performed, comparing the present method (y) to a commercially available direct LDL method (x) with the following results: y = 1.025x - 4.0289, r = 0.9969. Studies performed according to NCCLS Guideline, EP9-T.

Precision: Within-Day and Day-to-Day precision for the Direct HDL Cholesterol LiquiColor® method was determined following a modification of NCCLS document EP5-T2. Precision studies produced the following results:

Within-Day

Mean (mg/dL)	SD	CV%
50	0.28	0.56
99	0.43	0.44

Day-to-Day

Mean (mg/dL)	SD	CV%
97	1.29	1.33
137	1.92	1.40
204	2.90	1.43

Sensitivity: Based on an instrument resolution of A=0.001 absorbance units, the method presented shows a sensitivity of 0.4 mg/dL of LDL Cholesterol.

Linearity: When performed as directed this method is linear to 520 mg/dL. Performed according to NCCLS Guideline, EP6-P.

References

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10. Young D.S., Effects on Drugs in Clinical Laboratory Tests, 3rd ed., AACC Press, Washington DC, 1990, 3-104 thru 3-106.
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12. INTERCHIM Laboratory Data

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