DIRECT LDL CHOLESTEROL

LDL

REF	Kit Size	
BC101-002	R1: 4 x 45 mL	R2: 4 x 15 mL

Intended Use:

For the quantitative determination of the concentration of Low-Density Lipoprotein Cholesterol (LDL-C) in human serum or plasma.

Test Summary

Cholesterol is typically acquired through the absorption of dietary and biliary cholesterol in the intestines, but it can also be produced in various tissues, primarily in the liver and intestines. In adults following a low-cholesterol diet, the body typically generates about 800 mg of cholesterol daily. Cholesterol plays a vital role in all cells and serves as a critical structural component in cell membranes. Additionally, it acts as a building block for the synthesis of bile acids, vitamin D, and sex hormones, including estradiol, progesterone, androgens, and testosterone. Since cholesterol is not soluble in water, it needs to be transported while bound to proteins.

Lipoproteins are complex particles containing cholesterol esters and triglycerides in a central core, surrounded by free cholesterol, phospholipids, and apolipoproteins that aid in their formation and function. Plasma lipoproteins can be categorized into different classes based on size, lipid composition, and apolipoproteins. The four primary classes are chylomicrons, very low-density lipoproteins (VLDL), low-density lipoproteins (LDL), and high-density lipoproteins (HDL). LDL, which contains a significant amount of cholesterol and cholesterol esters, is derived from VLDL and IDL in the bloodstream. LDL's main role is to transport these forms of cholesterol to peripheral tissues, with the majority of circulating cholesterol residing in LDL.

Various sources of evidence from epidemiological, genetic, and clinical studies suggest that LDL plays a causative role in the development of atherosclerotic cardiovascular disease (ASCVD). Elevated LDL-C levels are a major risk factor for the formation of atherosclerotic plaques in arterial walls and are strongly associated with coronary heart disease (CHD) and related mortality. Recent clinical studies have indicated continued benefits from lowering LDL-C levels, and there is a direct linear relationship between the reduction of LDL-C through medications like statins, ezetimibe, and proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors, and the reduction in relative risk of cardiovascular events.

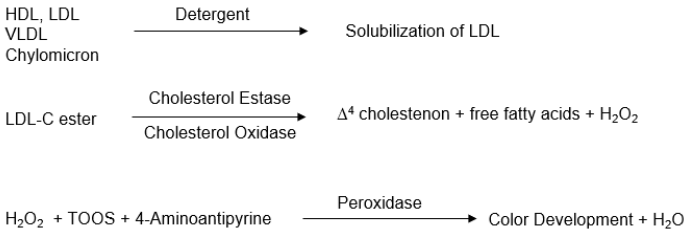
While the standard lipid panel is a well-established method for assessing risk, it may not be sufficient or fully informative. Most current screening guidelines recommend a comprehensive lipid profile assessment, which includes measurements of total cholesterol (TC), LDL-C, HDL-cholesterol (HDL-C), and triglycerides (TG).

Test principle

Various techniques are available for assessing LDL-C levels. The standard method involves ultracentrifugation, which is intricate and time-consuming, making it unsuitable for routine use.

In clinical laboratories, a common method for estimating LDL-C is the Friedewald calculation, which relies on measurements of total cholesterol (TC), triglycerides (TG), and HDL-C. However, this method only provides an approximation of LDL-C and has known limitations.

In recent years, new methods have been introduced that offer more convenient and automated ways to directly measure LDL-C. These homogeneous LDL-C methods allow for the direct determination of LDL-cholesterol and offer several advantages compared to earlier techniques. For example, the LDL-C direct method is a homogeneous approach that doesn't require centrifugation steps and enables the direct measurement of LDL-cholesterol. This method uses block polymer detergents to protect HDL, VLDL, and chylomicrons, ensuring that only LDL-cholesterol is selectively measured through enzymatic cholesterol assessment.



Reagents

Composition of Reagents

Constituents	**Reagent 1 (R1)** Good's buffer (pH 7.1) 4-Aminoantipyrine <0,01 % BSA <0,1 % TOOS <0,05% B66 <2,0 % Preservative <0,1 % Cholesterol Oxidase >500 U Esterase >100 U Peroxidase >500 U **Reagent 2 (R2)** Good's buffer (Ph 6.7) 4-aminoantipyrine <0,05 % Preservative <0,1 %

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Warnings and Precautions for Use

Reagent 1:

Warning Contains: Mixture of 5-chlorine-2-methyl-2H-isothiazol-3-on and 2-methylen-2H-isothiazol-3-on
Hazard statements
H317 - May cause an allergic skin reaction
Precautionary statements
P261 - Avoid breathing dust/fume/gas/mist/vapors/spray.
P272 - Contaminated work clothing must not be allowed out of the workplace.
P280 - Wear protective gloves/protective clothing/eye protection/face protection.
P302+P352 - If on skin: Wash with plenty of water.
P321 - Specific treatment (see supplemental first aid instruction on this label).
P333+P313 - If skin irritation or rash occurs: Get medical advice/attention.

P363 - Wash contaminated clothing before reuse.
P501 - Dispose of contents/container to hazardous or special waste collection point, in accordance with local, regional, national and/or international regulation.

Reagent 2:

Warning Contains: Mixture of 5-chlorine-2-methyl-2H-isothiazol-3-on and 2-methylen-2H-isothiazol-3-on
H317 - May cause an allergic skin reaction
Precautionary statements
P261 - Avoid breathing dust/fume/gas/mist/vapors/spray.
P272 - Contaminated work clothing must not be allowed out of the workplace.
P280 - Wear protective gloves/protective clothing/eye protection/face protection.
P302+P352 - If on skin: Wash with plenty of water.
P321 - Specific treatment (see supplemental first aid instruction on this label).
P333+P313 - If skin irritation or rash occurs: Get medical advice/attention.
P363 - Wash contaminated clothing before reuse.

P501 - Dispose of contents/container to hazardous or special waste collection point, in accordance with local, regional, national and/or international regulation.
Do not pipette by mouth.

All specimens used in the 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 the reagents after the expiration date printed on the reagent label.

Preparation of reagents and stability

Reagent 1: Ready to use.

Reagent 2: Ready to use.

Storage temperature: 2- 8°C

Shelf life: 15 months from the date of manufacturing

Disposal

Reagents must be disposed of in accordance with all Federal, Provincial, State, and local regulations.

Specimen

The required specimens for testing should be either serum or plasma obtained from the patient after a 12 to 14-hour fasting period.
For serum: Collect whole blood through venipuncture, allow it to clot, and then promptly centrifuge and separate the serum within 3 hours of collection.
For plasma: Specimens can be collected in EDTA, lithium, or sodium heparin. Centrifuge and separate the plasma as soon as possible within 3 hours of collection.
It's important to note that serum or plasma should not be left at temperatures between 18-28°C for more than 24 hours. If testing cannot be completed within this timeframe, store the serum or plasma at 2-8°C for up to 2 days. If longer storage is necessary, specimens can be preserved at temperatures below -20°C for up to a month, and samples can be frozen once. For more detailed instructions on specimen collection, handling, and storage, please refer to NCCLS Document H18-A.

Analytical Specificity

All interference studies were conducted according to a modified NCCLS guideline No. EP7 for interference testing in clinical chemistry.

Substances Tested	Concentration with no significant (±10%) interference
Hemoglobin	500 mg/dL
Triglycerides	3000 mg/dL
Bilirubin	20 mg/dL

Limitations

1. Anticoagulants containing citrate should not be used.
2. Protect the reagents from direct sunlight.
3. Store the reagents at 2-8°C. Do not freeze the reagents.

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4. The NCEP recommends that dietary and/or drug treatment not be based on a single LDL cholesterol result.
5. Endogenous triglyceride levels gave acceptable performance up to 3000 mg/dL. Samples with triglyceride level >3000 mg/dL should not be diluted.
6. Samples containing the following should not be used: N-acetylcysteine (NAC).

General precautions

1. This product is for *in vitro* diagnostic use and must not be used for any other purpose.
2. When a clinical decision is made based on the test results obtained by using Cholesterol LDL, the clinician should also take into consideration the patient's clinical condition and findings on examination.
3. This product should be used as directed in this package insert. The reliability of the measurement values cannot be guaranteed if Cholesterol LDL is used for purposes or tested by methods not stated in this document.
4. If the reagents contact with the eyes or mouth, rinse thoroughly with water as a first aid measure and consult a doctor if required.
5. Carefully read the operating instructions for each type of automated analyzer.
6. After calibration, perform bilevel quality assessment.

Analytical Procedure

Wavelength	546/700 nm
Temperature	+37°C
Measurement	Endpoint
Sample/Calibrator	10 µL
Reagent 1	900 µL
Reagent 2	300 µL
1st Incubation (R1+Sample)	5 minutes
2nd Incubation (After adding R2)	5 minutes
Calibration	Linear

Materials required but not provided

- LDL Calibrator
- LDL Control Level 1 & Level 2

*Any human cholesterol-based calibrator and control material can be used. After calibration, Quality Control values must be within the expected range. Quality control requirements should be established in accordance with local, state, and/or federal regulations or accreditation requirements.

**Performance studies were conducted with URIT 8210 test systems. Before measuring sample, please apply your own instrument operator's manual for analyzer specific procedures and for guidance in determining calibration frequency.

Calculation

$$\text{LDL-C [mg/dL]} = \frac{\Delta A \text{ Sample}}{\Delta A \text{ Cal}} \times \text{Conc. Cal [mg/dL]}$$

Conversion Factor

LDL-C [mg/dL] x 0.02586 = LDL-C [mmol/L]

Reference Intervals

LDL hypocholesterolemia[2)] is said to be present with levels of 40 mg/dl or lower.

National Cholesterol Education Program (NCEP) guidelines[2]

< 160 mg/dL Low LDL-Cholesterol (negative risk factor for CHD)

>160 mg/dL High LDL-Cholesterol (major risk factor for CHD)

Reportable Range

Linearity assessments were carried out using a human serum sample. The linearity simples were prepared via diluted human serum. JTC LDL kit has a linear response in the range of 4.0 mg/dL to 450 mg/dL, with a deviation from the linear trend of no more than 10%.

Patient samples with LDL cholesterol levels exceeding 450 mg/dL should be diluted with physiological saline before assaying. Multiply the result obtained from the manual dilution by the appropriate dilution factor.

Storage and Stability

Reagents are stable up to the date of expiry indicated on the kit, if stored at +2-8°C and contamination is avoided. Do not freeze and protect from the light.

Precision

Within-run precision for the JTC LDL Cholesterol method was determined using three levels of frozen pooled human serum. Each run consisted of twenty replicate samples. Within-run precision studies produced the following results on the URIT 8210 Analyzer:

Serum Pool	Low	Mid	High
n	20	20	20
Mean (mg/dL)	113	149	188
Standard Deviation	1.93	2.16	1.57
CV%	1.72	1.45	0.84

Between-run precision for the JTC LDL Cholesterol method was determined using three levels of frozen pooled human serum. For 5 days, two different run was performed in a day as duplicate. Within-run precision studies produced the following results on the URIT 8210 Analyzer:

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Serum Pool	Low	Mid	High
n	20	20	20
Mean (mg/dL)	112	149	188
Standard Deviation	2.24	2.23	1.79
CV%	2.01	1.49	0.95

Total error determination:

Total error serves as a comprehensive measure of an assay's overall analytical performance, encompassing both accuracy and precision. It can be calculated as the sum of % Bias and XXX times the Total Coefficient of Variation (%CV). The % Bias for the JTC LDL assay kit was determined through a linear regression formula, derived from a comparison with the Sekisui LDL assay kit. Below are the results of the total error analysis for the JTC LDL assay kit on the Urit automated analyzers at low, medium, and high LDL Cholesterol levels.

LDL cholesterol concentration	Bias%	Total CV%	Total Error
80	1.5%	2%	5.42%
105	2.5%	1.72%	5.87%
130	1.75%	0.95%	3.61%

Accuracy

The accuracy of the JTC LDL Cholesterol assay was confirmed through a comparison with the Designated Comparison Method for LDL cholesterol, yielding the following results:

Method	JTC LDL assay kit	Sekisui LDL assay kit
n	50	50
Mean(mg/dl)	99.5	99.25
Range (mg/dl)	4-450 mg/dl	6.6-992 mg/dl
Correlation coefficient	y=0.9966x+1.14 R^2 = 0.997	

Reproducibility

Coefficient of variation of ≤5% (within run)

Method comparison

A comparison of the human material with a commercial competitor kit product (x) gave the following result:

y = 0.99 x + 0.39; r = 0.996 Serum

Literature

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	Manufacturer
IVD	In vitro diagnostic medical device
REF	Catalog Number
	Exclamation Mark

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