

LDL Direct Liquid Select Cholesterol Reagent

INTENDED USE

For the direct, quantitative measurement of low-density lipoprotein cholesterol (LDL-C) concentration in human serum or plasma.

SUMMARY

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, while the inner core contains mostly esterified cholesterol and triglyceride. These particles serve to solubilize and transport cholesterol and triglyceride in the bloodstream.

The relative proportions of protein and lipid determine the density of these lipoproteins and provide a basis on which to begin their classification.¹ These classes are: chylomicrons, very-low density lipoprotein (VLDL), low-density lipoprotein (LDL) and high density lipoprotein (HDL). Numerous clinical studies have shown that the different lipoprotein classes have very distinct and varied effects on coronary heart disease risk.²⁻⁴ The studies all point to LDL cholesterol as the key factor in the pathogenesis of atherosclerosis and coronary artery disease (CAD)²⁻⁸, while HDL cholesterol has 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.⁹

PRINCIPLE

The LDL Direct Liquid Select Cholesterol assay is a homogeneous method for directly measuring LDL-C concentrations in serum or plasma, without the need for any off-line pretreatment or centrifugation steps.

The method is in a two reagent format and depends on the properties of a unique detergent. 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.

REAGENTS

Composition of Reagents:

Component	Ingredients	Concentration
Reagent 1	Buffer	<1.0%
	Detergent 1	<1500 U/L
	Cholesterol esterase (Pseudomonas sp.)	<1500 U/L
	Cholesterol oxidase (Cellulomonas sp.)	<1300 ppg U/L
	Peroxidase (Horseradish)	<0.1%
	4-Aminoantipyrine	<3000 U/L
	Ascorbic oxidase (Curcubita sp.)	
Preservative		
Reagent 2	Buffer (pH 6.3)	<1.0%
	Detergent 2	<1.0 mM
	N,N-bis (4-sulfobutyl) -m-toluidine, disodium (DSBmT)	
	Preservative	

Precautions and Warnings

1. For *In Vitro* diagnostic use.
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 reagents after the expiration date printed on the label.

Preparation

Reagent 1: Reagent 1 is ready to use as packaged.
Reagent 2: Reagent 2 is ready to use as packaged.

Storage and Stability

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

Once opened, Reagent 1 and Reagent 2 is stable for 4 weeks at 2-8°C.

Once opened Reagent 1 and Reagent 2 have 4 weeks on board stability when stored at 2-8°C.

DO NOT FREEZE

Indications of Deterioration

Inability to recover control values.
Presence of turbidity.

SPECIMEN COLLECTION AND PREPARATION

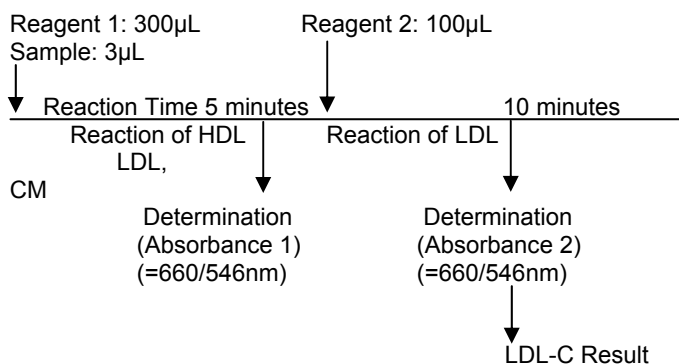
Patients are not required to fast prior to blood collection. Serum, EDTA-treated or heparinized plasma are the recommended specimens.

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

PROCEDURE

Assay

Below is a general example of the LDL Direct Liquid Select Cholesterol test procedure for a two reagent automated analyzer. All analyzer applications should be validated in accordance with NCEP and CLIA recommendations.^{10, 11} For assistance with applications on automated analyzers, please contact Genzyme Technical Marketing at 800-332-1042.



Materials Provided

Reagents and materials may be provided as follows:

Description	Configuration	Catalog Number
LDL Direct Liquid Select Cholesterol Reagent	R1 1 x 30 mL R2 1 x 10 mL	7120

Materials Required but not Provided

Description	Configuration	Catalog Number
N-geneous [®] LDL Cholesterol Calibrator	3 x 1 mL	80-4610-02

1. Class A volumetric pipettes.
2. Distilled, deionized, Type II water or equivalent.
3. Analyzer capable of running two reagent chemistries.

Calibration

The N-geneous[®] LDL Cholesterol Calibrator Kit is required for the calibration of this assay. Other commercially available LDL calibrators have not been tested with this assay and cannot be supported by Genzyme. The value of the LDL calibrator was assigned by procedures traceable to the CDC LDL cholesterol reference method.¹⁵ Refer to the instrument operator's manual for analyzer-specific calibration procedures and for guidance in determining calibration frequency.

Quality Control values should be within the expected range.

Quality Control

Reliability of test results should be routinely monitored with quality-control materials or serum that reasonably represent performance with patient specimens.¹⁰ Controls or serum pools should be run with each assay to ensure that the reagents are functioning properly and that correct procedures have been followed. An acceptable range for each lot of control material should be established by the laboratory. If control values are not within the expected range, confirm procedures were performed correctly and follow normal troubleshooting measures. If assistance is required, call Genzyme Technical Marketing 800-332-1042.

Quality control requirements should be established in accordance 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 0.02586.¹⁴

$$\text{mg/dL} \times 0.02586 = \text{mmol/L LDL-cholesterol}$$

Limitations / Interfering Substances

All interference studies were conducted according to NCCLS guideline No. EP7 for interference testing in clinical chemistry.¹²

Substance Tested

Concentration with no significant ($\pm 10\%$) interference

Ascorbic Acid	50 mg/dL
Hemoglobin	500 mg/dL
Bilirubin	20 mg/dL
Gamma-Globulins	5000 mg/dL

1. Refer to the work of Young for a review of drug interference on serum LDL cholesterol levels.¹³
2. Protect the reagent from direct sunlight.
3. Anticoagulants containing citrate should not be used.
4. Patient samples should only be frozen once.
5. Samples with triglyceride values up to 1,293mg/dL did not interfere with the results of the LDL Direct Liquid Select cholesterol assay. Samples with Triglyceride levels > 1,293 mg/dL should not be diluted.

Expected Values

The following NCEP cutpoints for patient classification are used for the prevention and management of coronary heart disease.⁸

It is recommended that each laboratory verify the reference interval for its patient population.

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

SPECIFIC PERFORMANCE CHARACTERISTICS

Accuracy

Accuracy of LDL Direct Liquid Select Cholesterol Reagent method was verified by comparison to the Reference Method (Ultracentrifugation and cholesterol analysis)¹⁰ and the Direct LDL Immunoseparation method.

Studies comparing the LDL Direct Liquid Select Cholesterol method to the Reference Method (Ultracentrifugation) produced the following results:

Method	LDL Direct Liquid Select Cholesterol	Reference Method
n	54	54
Mean LDL Cholesterol (mg/dL)	122.5	125.1
Standard Deviation (mg/dL)	30.7	30.9
Regression Analysis	$y = 0.95 + 3.02 \text{ mg/dL}$	
Correlation Coefficient	$r = 0.96$	

Studies comparing the LDL Direct Liquid Select Cholesterol method to the Direct LDL cholesterol Immunoseparation method produced the following results:

Method	LDL Direct Liquid Select Cholesterol	Reference Method
n	92	92
Mean LDL Cholesterol (mg/dL)	120.0	122.8
Standard Deviation (mg/dL)	30.5	31.6
Regression Analysis	$y = 0.94 + 4.46 \text{ mg/dL}$	
Correlation Coefficient	$r = 0.97$	

Precision

Within-run precision for the LDL Direct Liquid Select Cholesterol Reagent 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 Hitachi 911:

Serum Pool	LOW	MID	HIGH
n	20	20	20
Mean LDL Cholesterol (mg/dL)	98.1	146.5	209.8
Standard Deviation (mg/dL)	0.72	0.96	1.31
Coefficient of Variation (%)	0.73	0.66	0.62

Between-run precision was determined using three levels of frozen pooled human serum. The LDL Direct Liquid Select Cholesterol assay was run twice per day in duplicate over 10 days. Between-run precision studies produced the following results on the Hitachi 911:

Serum Pool	LOW	MID	HIGH
n	20	20	20
Mean LDL Cholesterol (mg/dL)	98.1	142.7	207.3
Standard Deviation (mg/dL)	2.2	2.8	3.6
Coefficient of Variation (%)	2.27	1.95	1.73

Limit of Detection

The limit of detection of the LDL Direct Liquid Select assay, quantified as 2 SDs plus the mean of twenty replicate measurements of saline, is 0.278 mg/dL on the Hitachi 911.

Linearity

The LDL Direct Liquid Select test method is linear from 6.6 mg/dL to 992 mg/dL.

REFERENCES

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Definitions for Symbols

REF

Catalog number

IVD

For *in vitro* diagnostic use



Temperature limitation



Manufactured by



Use by

LOT

Batch code



Consult instructions for use



Caution, consult accompanying document

Manufactured by:

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