DIRECT LDL

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Direct LDL

REF 1E31-20

307191R14

B1E310

FOR USE WITH

ARCHITECT

Package insert instructions must be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

Read Highlighted Changes: Revised February 2022.

INTENDED USE

The MULTIGENT Direct LDL assay is used for the direct, quantitative determination of low-density lipoprotein (LDL) cholesterol in human serum or plasma on the ARCHITECT *c*Systems.

SUMMARY AND EXPLANATION OF TEST

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 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 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 (CHD) risk.[2-4]

The studies all point to LDL cholesterol as the key factor in the pathogenesis of atherosclerosis and CHD,[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 CHD.[4]

PRINCIPLES OF PROCEDURE

The MULTIGENT Direct LDL assay is a homogeneous method for directly measuring LDL levels in serum or plasma, without the need for 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, R1, solubilizes only the non-LDL particles. The cholesterol released is consumed by cholesterol esterase and cholesterol oxidase in a non-color-forming reaction. A second detergent, R2, solubilizes the remaining LDL particles and a chromogenic coupler allows for color formation. The enzyme reaction with LDL in the presence of the coupler produces color that is proportional to the amount of LDL cholesterol present in the sample.

Methodology: Measured, Liquid Selective Detergent

REAGENTS

Reagent Kit

REF 1E31-20 MULTIGENT Direct LDL is supplied as a liquid, ready-to-use, two-reagent kit which contains:

- R1 2 x 53 mL
- R2 2 x 20 mL

Estimated tests per kit: 450
Calculation is based on the minimum reagent fill volume per kit.

Reactive Ingredients		Concentration
R1	MES buffer (pH 6.3)	
	Detergent 1	< 1.0%
	Cholesterol esterase (Microorganism)	< 1,500 U/L
	Cholesterol oxidase (Microorganism)	< 1,500 U/L
	Peroxidase (Horseradish)	< 1,300 ppg U/L
	4-aminoantipyrine	< 0.01%
	Ascorbic acid oxidase (Cucurbita sp.)	< 3,000 U/L
	Preservative	
R2	MES buffer (pH 6.3)	
	Detergent 2	< 1.0%
	N,N-bis(4-sulfobutyl)-m-toluidine, disodium (DSBmT)	< 1.0 mmol/L
	Preservative	

REAGENT HANDLING AND STORAGE

Reagent Handling

Remove air bubbles, if present in the reagent cartridge, with a new applicator stick. Alternatively, allow the reagent to sit at the appropriate storage temperature to allow the bubbles to dissipate. To minimize volume depletion, do not use a transfer pipette to remove the bubbles.

CAUTION: Reagent bubbles may interfere with proper detection of reagent level in the cartridge, causing insufficient reagent aspiration that could impact results.

Reagent Storage

Unopened MULTIGENT Direct LDL reagents are stable until the expiration date shown on the label when stored at 2 to 8°C. Do not freeze.

Reagent stability is 28 days (672 hours) if the reagent is uncapped and onboard.

Indications of Deterioration

Instability or deterioration should be suspected if there are precipitates, visible signs of leakage, extreme turbidity, microbial growth, if calibration does not meet the appropriate package insert and/or **ARCHITECT System Operations Manual** criteria, or if controls do not meet the appropriate criteria.

WARNINGS AND PRECAUTIONS

Precautions for Users

- IVD
- For *In Vitro* Diagnostic Use.
- Do not use reagents beyond the expiration date.
- Do not mix reagents from different kit lot numbers.
- Protect reagents from direct sunlight.
- Do not freeze reagents.
- **CAUTION:** This product requires the handling of human specimens. It is recommended that all human-sourced materials be considered potentially infectious and handled in accordance with the OSHA Standard on Bloodborne Pathogens.[9] Biosafety Level 2[10] or other appropriate biosafety practices[11,12] should be used for materials that contain or are suspected of containing infectious agents.

The following warnings and precautions apply to: R1 and R2	
	
WARNING:	Contains methylisothiazolones.
H317	May cause an allergic skin reaction.
Prevention	
P261	Avoid breathing mist / vapors / spray.
P272	Contaminated work clothing should not be allowed out of the workplace.
P280	Wear protective gloves / protective clothing / eye protection.
Response	
P302+P352	IF ON SKIN: Wash with plenty of water.
P333+P313	If skin irritation or rash occurs: Get medical advice / attention.
P362+P364	Take off contaminated clothing and wash it before reuse.
Disposal	
P501	Dispose of contents / container in accordance with local regulations.

Safety Data Sheets are available at www.abbottdiagnostics.com or contact your local representative.

For a detailed discussion of safety precautions during system operation, refer to the **ARCHITECT System Operations Manual,** *Section 8.*

SPECIMEN COLLECTION AND HANDLING

Suitable Specimens

Serum and plasma are acceptable specimens. Patients are not required to fast prior to blood collection.

- **Serum:** Use serum collected by standard venipuncture techniques into glass or plastic tubes with or without gel barriers. Ensure complete clot formation has taken place prior to centrifugation. Separate serum from red blood cells or gel as soon after collection as possible (within 3 hours).[13]

  Some specimens, especially those from patients receiving anticoagulant or thrombolytic therapy, may take longer to complete their clotting processes. Fibrin clots may subsequently form in these sera and the clots could cause erroneous results.
- **Plasma:** Use plasma collected by standard venipuncture techniques into glass or plastic tubes. Acceptable anticoagulants are lithium heparin (with or without gel barrier), sodium heparin, and EDTA. Anticoagulants containing citrate should not be used. Ensure centrifugation is adequate to remove platelets. Separate plasma from red blood cells or gel as soon after collection as possible (within 3 hours).[13]

Refer to the specimen collection tube manufacturer's instructions for processing and handling requirements.

For total sample volume requirements, refer to the ASSAY PARAMETERS section of this package insert and *Section 5* of the **ARCHITECT System Operations Manual**.

Specimen Storage

Serum and Plasma

Temperature	Maximum Storage
2 to 8°C	5 days
-80°C	3 months

Samples may be frozen once. Refer to Clinical and Laboratory Standards Institute (CLSI) document NCCLS H18-A3 for further instructions on specimen collection, handling, and storage.[14]

NOTE: Stored specimens must be inspected for particulates. If present, mix and centrifuge the specimen to remove particulates prior to testing.

PROCEDURE

Materials Provided

REF 1E31-20 MULTIGENT Direct LDL Reagent Kit

Materials Required but not Provided

- REF 5P56-01 or 5P56-02 Lipid Multiconstituent Calibrator (Lipid MCC)
- LDL cholesterol control sera or quality control material
- Saline (0.85% to 0.90% NaCl) for specimens that require dilution

Assay Procedure

For a detailed description of how to run an assay on the ARCHITECT *c* Systems, refer to *Section 5* of the **ARCHITECT System Operations Manual**.

Specimen Dilution Procedures

Serum and Plasma: The following specimens should be diluted by following the Manual Dilution Procedure:

- Specimens with levels of interfering substances (other than triglyceride) higher than the upper limit stated in the Interfering Substances section.
- Specimens with LDL cholesterol values exceeding 800 mg/dL (20.69 mmol/L).

Manual Dilution Procedure

Manual dilutions should be performed as follows:

- Use saline (0.85% to 0.90% NaCl) to dilute the sample.
- The operator must enter the manual dilution factor in the patient or control order screen. The system uses this dilution factor to automatically correct the concentration by multiplying the result by the entered factor.
- If the operator does not enter the dilution factor, the result must be multiplied by the appropriate dilution factor before reporting the result.

$$\text{Manual Dilution Factor} = \frac{(\text{Volume of Sample} + \text{Volume of Dilution Reagent})}{\text{Volume of Sample}}$$

NOTE: If a diluted sample result is flagged indicating it is less than the linear low limit, do not report the result. Rerun using an appropriate dilution.

For detailed information on ordering dilutions, refer to *Section 5* of the **ARCHITECT System Operations Manual**.

CALIBRATION

Calibration is stable for approximately 28 days (672 hours) and is required with each change in reagent lot number. Verify calibration curve with at least two levels of controls according to the established quality control requirements for your laboratory. If control results fall outside acceptable ranges, recalibration may be necessary.

For a detailed description of how to calibrate an assay, refer to *Section 6* of the **ARCHITECT System Operations Manual**.

For information on calibrator standardization, refer to the package insert for REF 5P56-01 Lipid Multiconstituent Calibrator.

QUALITY CONTROL

Reliability of test results should be routinely monitored with quality control materials or serum pools that reasonably represent performance on patient specimens.[15]

As appropriate, refer to your laboratory standard operating procedure(s) and/or quality assurance plan for additional quality control requirements and potential corrective actions.

- Run two levels of controls (normal and abnormal) once during each day of assay use.
- If more frequent control monitoring is required, follow the established quality control procedures for your laboratory.
- If quality control results do not meet the acceptance criteria defined by your laboratory, patient values may be suspect. Follow the established quality control procedures for your laboratory. Recalibration may be necessary.
- Review quality control results and acceptance criteria following a change of reagent or calibrator lot.

RESULTS

Refer to *Appendix C* of the **ARCHITECT System Operations Manual** for information on results calculations.

To convert results from mg/dL to mmol/L, multiply mg/dL by 0.02586.[16]

Representative performance data are given in the SPECIFIC PERFORMANCE CHARACTERISTICS section of this package insert. Results obtained in individual laboratories may vary.

LIMITATIONS OF THE PROCEDURE

Refer to the SPECIMEN COLLECTION AND HANDLING and SPECIFIC PERFORMANCE CHARACTERISTICS sections of this package insert.

EXPECTED VALUES

The following National Cholesterol Education Program (NCEP) cutpoints for patient classification are used for the prevention and management of coronary heart disease.[13]

Reference Range

LDL Cholesterol (mg/dL)	
< 100	Optimal
100 to 129	Near or above optimal
130 to 159	Borderline high
160 to 189	High
≥ 190	Very high

The NCEP Adult Treatment Panel III Report recommends the classification shown above. Laboratories should follow recommendations for lipid ranges effective in their locale if they differ from those of the NCEP.

SPECIFIC PERFORMANCE CHARACTERISTICS

Reportable Range

The reportable range of the MULTIGENT Direct LDL assay is from 1 to 800 mg/dL (0.03 to 20.69 mmol/L), based on limit of blank and linearity studies. Linearity was evaluated using N-geneous LDL Linearity Verifiers, part number 80-5953-00. Linearity samples recovered within 10% of the theoretical value up to 800 mg/dL.

Limit of Blank (LOB)

The LOB for MULTIGENT Direct LDL is ≤ 10 mg/dL (0.259 mmol/L). The LOB is the mean concentration of an analyte-free sample plus 2 SD, where SD = the pooled, within-run standard deviation of the analyte-free sample. A study performed on an ARCHITECT *c* System produced an LOB for MULTIGENT Direct LDL of 0.1 mg/dL (0.003 mmol/L).

Interfering Substances

Samples with triglyceride concentrations > 1,293 mg/dL (14.61 mmol/L) should not be used for the determination of LDL cholesterol.

Potential interference in the MULTIGENT Direct LDL assay from ascorbic acid, bilirubin, gamma globulins, and hemoglobin is less than 10% at the levels indicated below. A study based on guidance from NCCLS publication EP7-P[17] was performed using the MULTIGENT Direct LDL assay on a commercially available clinical chemistry analyzer. Varying amounts of potential interferents were added to serum pools with known quantities of cholesterol. No significant interference was detected in the MULTIGENT Direct LDL assay up to and including the concentrations stated below:

Interfering Substance	Concentration with No Significant Interference
Ascorbic acid	50 mg/dL (2,839 µmol/L)
Bilirubin (conjugated)	20 mg/dL (342 µmol/L)
Bilirubin (unconjugated)	20 mg/dL (342 µmol/L)
Gamma globulins	5,000 mg/dL (50 g/L)
Hemoglobin	500 mg/dL (5 g/L)

Interferences from medications or endogenous substances may affect results.[18]

The following drugs were tested for interference at the concentrations indicated using an acceptance criteria of ± 10% from the target value. Direct LDL is not affected by the presence of the following interferents up to and including the concentrations stated below:

Interfering Substance	Concentration with No Significant Interference
Acetaminophen	200 mg/L (1324.5 µmol/L)
Dipyrone	100 mg/L (300.3 µmol/L)
N-Acetyl-L-Cysteine	1600 mg/L (9816.0 µmol/L)

Precision

The precision of the MULTIGENT Direct LDL assay is < 4% CV. A within-run precision study was performed using two levels of LDL cholesterol control human serum. Each run consisted of 20 replicate samples.

In addition, a between-run precision study was performed using two levels of frozen pooled human serum in accordance with CLSI protocol NCCLS EP15-P.[19]

Representative data from these studies are summarized below.

	Control	Low < 130 mg/dL	High ≥ 160 mg/dL
	N	20	20
Within Run	Mean (mg/dL)	86.8	175.9
	SD	0.95	2.46
	%CV	1.1	1.4
Between Run	Mean (mg/dL)	89.0	178.1
	SD	1.92	3.94
	%CV	2.2	2.2

Accuracy

The average percent bias of the MULTIGENT Direct LDL reagent on a commercially available clinical chemistry analyzer to the Reference Method (ultracentrifugation and cholesterol analysis) was less than or equal to the guidelines established by the National Cholesterol Education Program (NCEP).[13] Fifty-four samples, with LDL values ranging from 68.1 to 214.5 mg/dL, were tested in duplicate using the LDL reagent on a commercially available clinical chemistry analyzer and the Reference Method. Sample means were compared by least squares linear regression analysis. Data from this study are summarized below.

Method	LDL	Reference Method
N	54	54
Mean (mg/dL)	122.5	125.1
Standard Deviation (mg/dL)	30.7	30.9
Regression Analysis	y = 0.95x + 3.02 mg/dL	
Correlation Coefficient	r = 0.96	

Method Comparison

The MULTIGENT Direct LDL assay on the AEROSET System was compared to a commercially available liquid selective detergent methodology. Samples were run in duplicate on both systems. Sample means were compared by least squares linear regression analysis. In a separate study, the MULTIGENT Direct LDL assay on an ARCHITECT *c* System was compared to the assay on the AEROSET System. Data from both studies are summarized below.

Method	AEROSET vs. Comparative Method	ARCHITECT vs. AEROSET
N	49	57
Y-Intercept	-1.60	-1.09
Correlation Coefficient	0.99	0.99
Slope	1.02	0.98
%Bias	0.6%	-2.9%
Range (mg/dL)	33.0 to 182.1	68.5 to 214.0

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TRADEMARKS

ARCHITECT *c* Systems Assay Parameters

Direct LDL Serum/Plasma—Conventional and SI Units

Configure assay parameters — General

● General O Calibration O SmartWash O Results O Interpretation

Assay: **DLDL** Type: **Photometric** Version: †
Number: **2840**
Run controls for onboard reagents by: **Lot**

● Reaction definition O Reagent / Sample O Validity checks

Reaction mode: **End up**
Primary Secondary Read times
Wavelength: **548** / **660** Main: **31** – **33**
Last required read: **33**
Absorbance range: ___ – ___ Color correction: ___ – ___
Sample blank type: **Self** Blank: **14** – **16**

O Reaction definition ● Reagent / Sample O Validity checks

						R1	R2
Reagent:	**DLDL0**			Reagent volume:		**200**	**67**
Diluent:	**Saline**			Water volume:		___	___
Diluent dispense mode:	**Type 0**			Dispense mode:		**Type 0**	**Type 0**

Dilution name	Sample	Diluted sample	Diluent	Water		Dilution factor	Default dilution
STANDARD :	**2.0**	___	___	___	=	1:1.00	●
_____ :	___	___	___	___	=		O
_____ :	___	___	___	___	=		O

O Reaction definition O Reagent / Sample ● Validity checks

Reaction check: **None**

Maximum absorbance variation: ___

Configure assay parameters — Calibration

O General ● Calibration O SmartWash O Results O Interpretation

Assay: **DLDL** Calibration method: **Linear**

● Calibrators O Volumes O Intervals O Validity checks

Calibrator set: **LIPIDMCC**
Calibrator level: Concentration:
Blank: **Water** **0**††
Cal 1: **LIPIDMCC1** ‡
Replicates: **3** (Range 1–3)

O Calibrators ● Volumes O Intervals O Validity checks

Calibrator: **LIPIDMCC**

Calibrator level	Sample	Diluted sample	Diluent	Water
Blank: **Water**	**2.0**	___	___	___
Cal 1: **LIPIDMCC1**	**2.0**	___	___	___

O Calibrators O Volumes ● Intervals O Validity checks

Calibration intervals:
Full interval: **672** (hours)
Calibration type:
Adjust type: **None**

O Calibrators O Volumes O Intervals ● Validity checks

Blank absorbance range: _____ – _____
Span: **Blank** – **Blank**
Span absorbance range: _____ – _____
Expected cal factor: **0.00**
Expected cal factor tolerance %: **0**

Configure assay parameters — SmartWash

O General O Calibration ● SmartWash O Results O Interpretation

Assay: **DLDL**

COMPONENT	REAGENT / ASSAY	WASH	Volume	Replicates

Direct LDL Serum/Plasma—Conventional Units

Configure assay parameters — Results

O General O Calibration O SmartWash ● Results O Interpretation

Assay: **DLDL** Assay number: **2840**
Dilution default range: Result units: **mg/dL**
Low-Linearity: **1**
High-Linearity: **800**
Gender and age specific ranges:**

GENDER	AGE (UNITS)	NORMAL	EXTREME
Either	**0 – 130 (Y)**	**100 – 159**	

Configure result units

Assay: **DLDL**
Version: †
Result units: **mg/dL**
Decimal places: **0** (Range 0–4)
Correlation factor: **1.0000**
Intercept: **0.0000**

Direct LDL Serum/Plasma—SI Units

Configure assay parameters — Results

O General O Calibration O SmartWash ● Results O Interpretation

Assay: **DLDL** Assay number: **2840**
Dilution default range: Result units: **mmol/L**
Low-Linearity: **0.03**
High-Linearity: **20.69**
Gender and age specific ranges:**

GENDER	AGE (UNITS)	NORMAL	EXTREME
Either	**0 – 130 (Y)**	**2.59 – 4.11**	

Configure result units

Assay: **DLDL**
Version: †
Result units: **mmol/L**
Decimal places: **2** (Range 0–4)
Correlation factor: **1.0000**
Intercept: **0.0000**

† Due to differences in instrument systems and unit configurations, version numbers may vary.
†† Displays the number of decimal places defined in the decimal places parameter field.
‡ Refer to the value on the calibrator labeling. These values are defined on the Configure calibrator set screen.
** User defined.

Key to Symbols

DISTRIBUTED BY	Distributed by
DISTRIBUTED IN THE USA BY	Distributed in the USA by
EC REP	Authorized Representative in the European Community
FOR USE WITH	Identifies products to be used together
INFORMATION FOR USA ONLY	Information needed for United States of America only
IVD	*In Vitro* Diagnostic Medical Device
LOT	Batch code/Lot number
PRODUCT OF CANADA	Product of Canada
R1	Reagent 1
R2	Reagent 2
REF	Catalog number/List number
SN	Serial number
WARNING: SENSITIZER	Warning: May cause an allergic reaction
	Consult instructions for use
	Manufacturer
	Sufficient for
	Temperature limitation
	Use by/Expiration date

Customer Service: Contact your local representative or find country-specific contact information on www.abbottdiagnostics.com.

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