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ADVIA® Chemistry XPT
Systems

LDL Cholesterol Direct (DLDL)

Current Revision and Date[a]	Rev. G, 2020-02	
Product Name	ADVIA® Chemistry LDL Cholesterol Direct (DLDL) Reagents	REF 09796248 (B01-4760-01)
Systems	ADVIA Chemistry XPT System	
Materials Required but Not Provided	ADVIA Chemistry HDL/LDL Cholesterol Calibrator Reagent container adapters Commercially available controls	REF 00309530 (B03-4763-01)
Specimen Types	Human serum, plasma (lithium heparin)	
Assay Principle	Elimination/catalase	
Assay Range	Serum: 8.0–1000.0 mg/dL (0.21–25.90 mmol/L) Plasma: 8.0–1000.0 mg/dL (0.21–25.90 mmol/L)	
Reagent Storage	2–8°C	
Reagent On-System Stability	14 days	
Reagent Code	74028	

[a] In Rev. E or later, a vertical bar in the margin indicates a technical update to the previous version.

Intended Use

For *in vitro* diagnostic use in the quantitative determination of LDL cholesterol in human serum or plasma on ADVIA® Chemistry XPT systems. Such measurements are used in the risk assessment of coronary artery disease.

Summary and Explanation

The ADVIA Chemistry LDL Cholesterol Direct (DLDL) assay measures LDL cholesterol in serum and plasma. The first step of the reaction eliminates cholesterol associated with lipoproteins other than low-density lipoprotein. A selective surfactant releases cholesterol preferentially from non-LDL particles.

Hydrogen peroxide produced by cholesterol esterase and cholesterol oxidase in the first step is eliminated by catalase. Another surfactant in DLDL Reagent 2 releases cholesterol from the low-density lipoprotein. Azide in ADVIA Chemistry DLDL Reagent 2 inhibits the catalase. Hydrogen peroxide generated by cholesterol esterase and cholesterol oxidase is quantified using a Trinder endpoint.[1]

Principles of the Procedure

The assay consists of 2 distinct reaction steps:

1. Cholesterol esterase and cholesterol oxidase eliminate cholesterol, other than from low density lipoprotein. The action of catalase removes the peroxide produced by the oxidase.

$$\text{Cholesterol ester} \xrightarrow{\text{Cholesterol esterase}} \text{Cholesterol+ Fatty acid}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{Cholesterol oxidase}} \text{Cholestenone} + H_2O_2$$

2. Specific measurement of LDL Cholesterol is made after its release by detergent in DLDL Reagent 2. Catalase from step 1 is inhibited by sodium azide in DLDL Reagent 2. The intensity of the quinoneimine produced in the Trinder reaction is directly proportional to the cholesterol concentration when measured at 596 nm.

$$\text{Cholesterol ester} \xrightarrow{\text{Cholesterol esterase}} \text{Cholesterol} + \text{Fatty acid}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{Cholesterol oxidase}} \text{Cholestenone} + H_2O_2$$

$$H_2O_2 + \text{4-Aminoantipyrine} + \text{TOOS}^{a} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine} + 4H_2O$$

[a] TOOS = N-ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methylaniline

Reagents

Reagent	Description	Storage	Reagent Stability
REF 09796248 (B01-4760-01)	**ADVIA Chemistry LDL Cholesterol Direct (DLDL) Reagents**		
LDL Cholesterol Direct Reagent 1 DLDL R1	19.2 mL in 20-mL containers PIPES buffer, pH 7.0 (50 mmol/L) N-ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methylaniline [TOOS] (2.0 mmol/L) Cholesterol esterase (E.C.3.1.1.13. *Pseudomonas*, 37°C) (≥ 600 U/L) Cholesterol oxidase (E.C.1.1.3.6. Nocardia, 37°C) (≥ 500 U/L) Catalase (E.C.1.11.1.6. microbial source, 25°C) (≥ 600 kU/L)	2–8°C	**Unopened:** Stable until the expiration date on product. **On-system:** 14 days
LDL Cholesterol Direct Reagent 2 DLDL R2	8.2 mL in 20-mL containers Goods buffer, pH 7.0 (50 mmol/L) 4-Aminoantipyrine (4 mmol/L) Peroxidase (E.C.1.11.1.7. Horseradish, 25°C) (≥ 4 kU/L) NaN_3 (0.09%)	2–8°C	**Unopened:** Stable until the expiration date on product. **On-system:** 14 days

Warnings and Precautions

Safety data sheets (MSDS/SDS) available on siemens.com/healthcare.

Caution

This device contains material of animal origin and should be handled as a potential carrier and transmitter of disease.

Contains sodium azide as a preservative. Sodium azide can react with copper or lead plumbing to form explosive metal azides. On disposal, flush reagents with a large volume of water to prevent the buildup of azides. Disposal into drain systems must be in compliance with prevailing regulatory requirements.

Dispose of hazardous or biologically contaminated materials according to the practices of your institution. Discard all materials in a safe and acceptable manner, and in compliance with prevailing regulatory requirements.

Caution: Federal (USA) law restricts this device to sale by or on the order of a licensed healthcare professional.

For *in vitro* diagnostic use.

Preparing Reagents

All reagents are liquid and ready to use.

Before use, gently invert the capped reagent to disrupt bubbles and ensure homogeneity. If bubbles still exist or foam is present, use a clean transfer pipette to aspirate them from the reagent container prior to use.

Storing and Stability

Unopened reagents are stable until the expiration date printed on the product label when stored at 2–8°C. Do not freeze reagents. Protect reagents from light.

Specimen Collection and Handling

Siemens Healthcare Diagnostics validated serum and plasma (lithium heparin) for the ADVIA Chemistry DLDL assay.

Follow these guidelines for specimens used for this assay:

- Serum and plasma can be collected using recommended procedures for collection of diagnostic blood specimens by venipuncture.[2] Follow the instructions provided with your specimen collection device for use and processing.[3]
- Complete clot formation should take place before centrifugation.
- Serum or plasma should be physically separated from cells as soon as possible with a maximum limit of 2 hours from the time of collection.[4]
- Specimens should be free of particulate matter.
- Specimens should be as fresh as possible.

The purpose of handling and storage information is to provide guidance to users. It is the responsibility of the individual laboratory to use all available references and/or its own studies when establishing alternate stability criteria to meet specific needs.

Procedure

Materials Provided

The following materials are provided:

Item	Contents	Number of Tests
REF 09796248 (B01-4760-01)	Reagent 1: 4 × 20-mL containers Reagent 2: 4 × 20-mL containers	4 × 188

Materials Required but Not Provided

The following materials are required to perform this assay, but are not provided:

Item	Description
REF 00309530 (B03-4763-01)	ADVIA Chemistry HDL/LDL Cholesterol Calibrator
REF 10316975	20-mL reagent container adapter for 40-mL slot
REF 10723030	20-mL reagent container adapter for 70-mL slot
	Commercially available control materials

Assay Procedure

Sampling, reagent delivery, mixing, and processing are automatically performed by the ADVIA Chemistry system.

For detailed information on performing the procedure, refer to the system operating instructions.

Preparing the System

For detailed information on preparing the system, refer to the system operating instructions.

Preparing the Samples

Before placing samples on the system, ensure that samples have the following characteristics:

- Samples are free of fibrin or other particulate matter.
- Samples are free of bubbles.

On-System Stability

The ADVIA Chemistry DLDL reagents are stable on the system for 14 days.

Do not use reagents beyond the expiration date.

Performing Calibration

To calibrate the ADVIA Chemistry DLDL assay, use the ADVIA Chemistry HDL/LDL Cholesterol Calibrator (REF 00309530 (B03-4763-01)).

Enter the lot-specific calibrator values that are provided with each lot of calibrator. Perform the calibration as described in the calibrator instructions for use.

Calibration Frequency

Calibrate the assay every 14 days.

Calibrate the assay after the following events:

- When the reagent lot number changes
- When a reagent pack is replaced by a new reagent pack with the same lot number, and the previous reagent pack was recalibrated during use
- When a reagent pack is replaced by a new reagent pack with the same lot number, and an additional reagent blank was run on the previous reagent pack during use
- After replacing critical optical or hydraulic components
- When indicated by quality control procedures

Individual laboratory quality control programs and procedures may require more frequent calibration.

Reagent Blank (RBL) Frequency

The ADVIA Chemistry system measures the RBL during assay calibration.

Note Use deionized water as the sample for the RBL in the ADVIA Chemistry DLDL assay.

Performing Quality Control

Follow government regulations or accreditation requirements for quality control frequency.

At least once each day of use, analyze 2 levels (low and high) of a commercially available quality control (QC) material with known LDL cholesterol concentrations.

A satisfactory level of performance is achieved when the analyte values obtained are within the expected control range for the system or within your range, as determined by an appropriate internal laboratory quality control scheme.

The actual frequency of control in a laboratory is based on many factors, such as workflow, system experience, and government regulation. Each laboratory should evaluate the controls based on the frequency established by their laboratory guidelines.

Also, assay controls under the following conditions:

- Whenever you use a new reagent lot
- Following any system maintenance, cleaning, or troubleshooting procedure
- After performing a new calibration or an additional reagent blank

Follow your laboratory internal QC procedures if the results obtained are outside acceptable limits.

Taking Corrective Action

If the quality control results do not fall within the expected control range or within the laboratory's established values, do not report results. Take the following actions:

1. Determine and correct the cause of the unacceptable control results:
    a. Verify that the assay was performed according to the instructions for use.
    b. Verify that the materials are not expired.
    c. Verify that required maintenance was performed.
    d. Rerun the assay with fresh quality control samples, and confirm that quality control results are within acceptable limits before running patient samples.
    e. If the quality control results are not within acceptable limits, recalibrate the assay, and repeat the prior step.
    f. If necessary, contact your local technical support provider or distributor for assistance.
2. After corrective action is complete, repeat required testing of patient samples before reporting results.

Perform corrective actions in accordance with your established laboratory protocol.

Results

Calculation of Results

The system calculates and reports results based on the absorbance measurements of the test sample during the test, and of the calibrator(s) from calibration.

The instrument calculates the concentration of LDL cholesterol in mg/dL (common units) or mmol/L (SI units).

Conversion factor: mg/dL × 0.0259 = mmol/L

Interpretation of Results

Results of this assay should always be interpreted in conjunction with the patient's medical history, clinical presentation, and other findings.

Limitations

A number of substances cause physiological changes in serum, plasma, or urine analyte concentrations. A comprehensive discussion of possible interfering substances, their serum, plasma, or urine concentrations, and their possible physiological involvements is beyond the scope of this document. Consult the listed reference for specific details on known potential interfering substances.[5]

As with any chemical reaction, you must be alert to the possible effect on results of unknown interferences from medications or endogenous substances. The laboratory and physician must evaluate all patient results in light of the total clinical status of the patient.

Venipuncture should occur prior to Metamizole (Sulpyrine) administration due to the potential for falsely depressed results.

Expected Values

The reference ranges for serum and plasma for LDL cholesterol are listed in the following table.[6]

Sample	Reference Range
Optimal	< 100.0 mg/dL (< 2.60 mmol/L)
Near optimal/above optimal	100.0–129.0 mg/dL (2.60–3.30 mmol/L)
Borderline high	130.0–159.0 mg/dL (3.40–4.10 mmol/L)
High	160.0–189.0 mg/dL (4.10–4.90 mmol/L)
Very High	≥ 190.0 mg/dL (≥ 4.90 mmol/L)

Siemens provides this information for reference. As with all *in vitro* diagnostic assays, each laboratory should determine its own reference ranges for the diagnostic evaluation of patient results. Consider these values as a guideline only.

Performance Characteristics

Analytical Measuring Range

This assay is linear from 8.0–1000.0 mg/dL (0.21–25.90 mmol/L) for serum and plasma.

Results that are below the low end of the assay range are flagged **< Conc Range**. The test result is reported as < 8.0 mg/dL (< 0.21 mmol/L) for serum and plasma.

Results that are above the high end of the assay range are flagged **> Conc Range**.

Extended Measuring Range

Siemens has validated an automatic rerun condition for this assay that extends the reportable range to 1670.0 mg/dL (43.25 mmol/L) for serum and plasma. You may configure the system to trigger automatic reruns. Rerun results will be flagged **Autorepeat**.

Sensitivity

The ADVIA Chemistry DLDL assay performance at low levels was analyzed as described in CLSI Document EP17-A2, and the limit of blank (LoB) and limit of detection (LoD) were determined.[7]

The LoB is the highest measurement result that is likely to be observed on a blank sample. The LoB for the ADVIA Chemistry DLDL assay is 0.1 mg/dL (0.00 mmol/L) (rounded to reportable digits).

The LoD is the smallest amount that this assay can reliably detect to determine presence or absence of an analyte. The LoD for the ADVIA Chemistry DLDL assay is 8.0 mg/dL (0.21 mmol/L).

The LoB and LoD values are determined with proportions of false positives (α) less than 5% and false negatives (β) less than 5%, based on 180 determinations with 120 blank and 60 low-level sample replicates.

Precision

The precision of the ADVIA Chemistry DLDL assay was analyzed as described in CLSI protocol EP05-A2.[8] Each sample was assayed 3 times per run, 2 runs per day, for at least 10 days.

Precision: Common Units

			Repeatability (Within-Run)		Between-Run		Between-Day		Within-Lab (Total)	
Specimen Type	N	Mean (mg/dL)	SD[a] (mg/dL)	CV[b] (%)	SD (mg/dL)	CV (%)	SD (mg/dL)	CV (%)	SD (mg/dL)	CV (%)
Serum Control 1	60	54.3	0.20	0.4	0.24	0.4	0.79	1.5	0.85	1.6
Serum Control 2	60	94.2	0.43	0.5	0.70	0.7	1.60	1.7	1.80	1.9
Serum Control 3	60	140.5	0.71	0.5	1.09	0.8	1.99	1.4	2.37	1.7
Serum Pool 1	60	317.3	1.13	0.4	2.11	0.7	3.68	1.2	4.39	1.4

[a] SD = standard deviation
[b] CV = coefficient of variation

Precision: SI Units

			Repeatability (Within-Run)		Between-Run		Between-Day		Within-Lab (Total)	
Specimen Type	N	Mean (mmol/L)	SD[a] (mmol/L)	CV[b] (%)	SD (mmol/L)	CV (%)	SD (mmol/L)	CV (%)	SD (mmol/L)	CV (%)
Serum Control 1	60	1.41	0.005	0.4	0.006	0.4	0.020	1.5	0.022	1.6
Serum Control 2	60	2.44	0.011	0.5	0.018	0.7	0.041	1.7	0.047	1.9
Serum Control 3	60	3.64	0.018	0.5	0.028	0.8	0.052	1.4	0.061	1.7
Serum Pool 1	60	8.22	0.029	0.4	0.055	0.7	0.095	1.2	0.114	1.4

[a] SD = standard deviation
[b] CV = coefficient of variation

Actual results will vary depending on the study design, and on the sample and sample population used. Results obtained at individual laboratories may vary from the data provided.

Accuracy / Method Comparison

The performance of the ADVIA Chemistry DLDL assay (y) was compared with the performance of the comparison assay on the indicated system (x).

Specimen Type	Comparison Assay (x)	N	r	Regression Equation	Sy.x	Sample Range
Serum	ADVIA 2400 DLDL	129	1.000	y = 0.99x - 6.74 mg/dL y = 0.99x - 0.175 mmol/L	5.79 mg/dL 0.150 mmol/L	22.6–987.0 mg/dL 0.59–25.56 mmol/L
Serum	ADVIA 1800 DLDL	129	0.999	y = 1.00x - 10.85 mg/dL y = 1.00x - 0.281 mmol/L	7.01 mg/dL 0.182 mmol/L	22.6–956.8 mg/dL 0.59–24.78 mmol/L
Plasma (Lithium Heparin)	ADVIA 1800 DLDL	53	1.000	y = 1.01x - 4.35 mg/dL y = 1.01x - 0.113 mmol/L	5.98 mg/dL 0.155 mmol/L	21.2–951.4 mg/dL 0.55–24.64 mmol/L
Plasma[a] (Lithium Heparin) ADVIA 1650/1800	ADVIA 1650/1800 DLDL - Serum	25	0.987	y = 1.02x - 0.8 mg/dL y = 1.02x - 0.02 mmol/L	3.5 mg/dL 0.09 mmol/L	73–147 mg/dL 1.9–3.8 mmol/L

[a] Matrix comparison. Correlations between serum and plasma samples on ADVIA 1650/1800 Chemistry systems are provided for reference.

The correlation of the assay may vary depending on the study design, comparable method, and sample population. Results obtained at individual laboratories may vary from the data provided.

Interferences

Siemens tested the following potential interferents and found the results shown below.

Interferent	Interferent Level	LDL Cholesterol Sample Concentration	Interference
Bilirubin (conjugated)	60 mg/dL (1026 µmol/L)	106.9 mg/dL (2.77 mmol/L)	NSI[a]
	60 mg/dL (1026 µmol/L)	158.0 mg/dL (4.09 mmol/L)	NSI
Bilirubin (unconjugated)	60 mg/dL (1026 µmol/L)	107.0 mg/dL (2.77 mmol/L)	NSI
	60 mg/dL (1026 µmol/L)	158.6 mg/dL (4.11 mmol/L)	NSI
Hemolysis (hemoglobin)	1000 mg/dL (10.0 g/L)	108.0 mg/dL (2.80 mmol/L)	NSI
	1000 mg/dL (10.0 g/L)	159.3 mg/dL (4.13 mmol/L)	NSI
Lipemia[b] (triglycerides from Intralipid)	1000 mg/dL (11.3 mmol/L)	108.1 mg/dL (2.80 mmol/L)	NSI
	750 mg/dL (8.5 mmol/L)	160.0 mg/dL (4.14 mmol/L)	NSI
	1000 mg/dL (11.3 mmol/L)	160.0 mg/dL (4.14 mmol/L)	-10.2%

[a] NSI = No significant interference. A percentage effect ≥ 10% is considered a significant interference.

[b] SI units calculated as triolein

Note There is poor correlation between turbidity and triglyceride concentration in a lipemic sample.[9]

Actual results will vary depending on the study design, the levels of the potential interferences tested, and the samples used. Results obtained at individual laboratories may vary from the data provided.

Standardization

The ADVIA Chemistry DLDL assay is traceable to the CDC Reference Method via patient sample correlation. Assigned values of ADVIA Chemistry HDL/LDL Cholesterol Calibrators are traceable to this standardization.

Technical Assistance

For customer support, please contact your local technical support provider or distributor.

siemens.com/healthcare

References

1. Izawa S, Okada M, Matsui H, *et al*. Low density lipoprotein cholesterol can be chemically measured: A new superior method. *Medicine and Pharmaceutical Sci.* 1997;37:1385–1388.
2. Clinical and Laboratory Standards Institute (formerly NCCLS). *Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture; Approved Guideline—Sixth Edition.* CLSI document GP41-A6. Wayne, PA: Clinical and Laboratory Standards Institute; 2008.
3. Clinical and Laboratory Standards Institute (formerly NCCLS). *Tubes and Additives for Venous Blood Specimen Collection: Approved Standard; Approved Guideline—Sixth Edition.* CLSI document GP39-A6. Wayne, PA: Clinical and Laboratory Standards Institute; 2010.
4. Clinical and Laboratory Standards Institute (formerly NCCLS). *Procedures for the Handling and Processing of Blood Specimens; Approved Guideline—Fourth Edition.* CLSI document GP44-A4. Wayne, PA: Clinical and Laboratory Standards Institute; 2010.
5. Young DS. *Effects of Drugs on Clinical Laboratory Tests.* 5th ed. Washington, DC: AACC Press; 2000.
6. *Third Report of the Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III)*, NIH Publication No. 01-3670, May 2001.
7. Clinical and Laboratory Standards Institute (formerly NCCLS). *Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline—Second Edition.* CLSI document EP17-A2. Wayne, PA: Clinical and Laboratory Standards Institute; 2012.
8. Clinical and Laboratory Standards Institute (formerly NCCLS). *Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline—Second Edition.* CLSI document EP05-A2. Wayne, PA: Clinical and Laboratory Standards Institute; 2004.
9. Twomey PJ, Don-Wauchope AC, McCullough D. Unreliability of triglyceride measurement to predict turbidity induced interference. *J Clin Pathol.* 2003 Nov;56(11):861–862.

Definition of Symbols

The following symbols may appear on the product labeling:

Symbol	Definition	Symbol	Definition
IVD	*In vitro* diagnostic medical device	REF REF	Catalog number
	Legal manufacturer	EC REP	Authorized Representative in the European Community
CE	CE Mark	CE 0088	CE Mark with identification number of notified body
	Consult instructions for use		Biological risk
	Keep away from sunlight and heat		Temperature limitation
	Lower limit of temperature		Upper limit of temperature
	Do not freeze (> 0°C)		Up
	Use by	Σ (n)	Contains sufficient for (n) tests
	Recycle	PRINTED WITH SOY INK	Printed with soy ink
Rev.	Revision	YYYY-MM-DD	Date format (year-month-day)
LOT	Batch code	**RxOnly**	Prescription Device (US only)

Trademarks

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