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ADVIA® Chemistry XPT
Systems

Cholesterol_2 (CHOL_2)

Current Revision and Date[a]	Rev. D, 2016-09	
Product Name	ADVIA® Chemistry Cholesterol_2 (CHOL_2) Reagent	REF 10376501
Systems	ADVIA Chemistry XPT System	
Materials Required but Not Provided	Siemens Chemistry Calibrator Reagent container adapters Commercially available controls	REF 09784096 (T03-1291-62)
Specimen Types	Human serum, plasma (lithium heparin)	
Assay Principle	Enzymatic	
Assay Range	Serum: 10–675 mg/dL (0.26–17.48 mmol/L) Plasma: 10–675 mg/dL (0.26–17.48 mmol/L)	
Reagent Storage	2–8°C	
Reagent On-System Stability	60 days	
Reagent Code	74719	

[a] In Rev. C 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 cholesterol in human serum or plasma on ADVIA® Chemistry XPT systems. Such measurements are used in the diagnosis and treatment of disorders involving excess cholesterol in the blood and in lipid and lipoprotein metabolism disorders.

Summary and Explanation

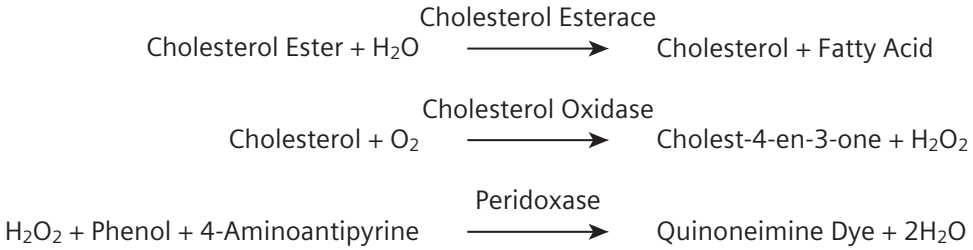
In the early 1980s, findings from the Coronary Primary Prevention Trial first demonstrated that lowering of a subject's plasma cholesterol level reduces the incidence of coronary heart disease. Also, cholesterol is associated with a variety of disorders that result from hyperlipidemia and dyslipoproteinemia.

The ADVIA Chemistry Cholesterol_2 (CHOL_2) assay is based on an enzymatic method using cholesterol esterase and cholesterol oxidase conversion followed by a Trinder endpoint.[1,2,3,4,5]

Principles of the Procedure

The cholesterol esters are hydrolyzed by cholesterol esterase to cholesterol and free fatty acids. The cholesterol is converted to cholest-4-en-3-one by cholesterol oxidase in the presence of oxygen to form hydrogen peroxide. A colored complex is formed from hydrogen peroxide, 4-aminoantipyrine and phenol under the catalytic influence of peroxidase. The absorbance of the complex is measured as an endpoint reaction at 505/694 nm.

Reaction Equation



Reagents

Reagent	Description	Storage	Reagent Stability
REF 10376501	**ADVIA Chemistry Cholesterol_2 (CHOL_2) Reagent**		
Cholesterol_2 Reagent 1 CHOL_2 R1	38 mL in 40-mL containers 4-Aminoantipyrine (0.25 mmol/L) Phenol (6.00 mmol/L) Peroxidase (horseradish) (≥ 0.50 U/mL) Cholesterol Esterase (*Pseudomonas*) (≥ 0.20 U/mL) Cholesterol Oxidase (*Nocardia*) (≥ 0.10 U/mL) NaN_3 (0.09%)	2–8°C	**Unopened:** Stable until the expiration date on product. **On-system:** 60 days

Warnings and Precautions

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

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.

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.

Specimen Collection and Handling

Siemens Healthcare Diagnostics validated serum and plasma (lithium heparin) for the ADVIA Chemistry CHOL_2 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.[6] Follow the instructions provided with your specimen collection device for use and processing.[7]
- 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.[8]
- 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 10376501	Reagent 1: 7 × 40-mL containers	7 × 350

Materials Required but Not Provided

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

Item	Description
REF 09784096 (T03-1291-62)	Siemens Chemistry Calibrator
REF 10719152	40-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 XPT 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 CHOL_2 reagents are stable on the system for 60 days.

Do not use reagents beyond the expiration date.

Performing Calibration

To calibrate the ADVIA Chemistry CHOL_2 assay, use the Siemens Chemistry Calibrator (REF 09784096 (T03-1291-62)).

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 60 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
- 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 XPT system measures the RBL during assay calibration.

Run an additional RBL on the same reagent pack every 7 days.

Run an additional RBL when a reagent pack is replaced by a new reagent pack with the same lot number, and an additional reagent blank was run during use.

Note Use deionized water as the sample for the RBL in the ADVIA Chemistry CHOL_2 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 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 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

United States federal law restricts this device to sale by or on the order of a physician.

A number of substances cause physiological changes in serum or plasma analyte concentrations. A comprehensive discussion of possible interfering substances, their serum or plasma 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.[9]

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.

Note Siemens has determined that there is a possibility for certain reagents to interact with the ADVIA Chemistry CHOL_2 assay when run on the same system. To mitigate these carryover events, the ADVIA Chemistry system software provides a Contamination Avoidance process. For further information and instructions to establish this process on your systems, refer to the Customer Bulletin entitled: *Consolidated Directory of Contamination Avoidance Settings for ADVIA Chemistry XPT Systems* (PN 10815606, latest revision).

Venipuncture should occur prior to N-Acetyl Cysteine (NAC) or Metamizole (Sulpyrine) administration due to the potential for falsely depressed results.

Expected Values

Guidelines for reference ranges have been suggested by the United States Panel of the National Institute of Health's Cholesterol Consensus Development Conference and adopted by the National Cholesterol Education Program. Consult your local accrediting agency, the College of American Pathologists, or the Joint Commission on Accreditation of Hospitals for guidance.

The suggested guidelines of the panel are:[10]

Risk Level	Total Cholesterol
Low (desirable)	< 200 mg/dL (< 5.18 mmol/L)
Moderage (borderline)	200–239 mg/dL (5.18–6.19 mmol/L)
High	≥ 240 mg/dL (≥ 6.22 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 10–675 mg/dL (0.26–17.48 mmol/L).

Results that are below the assay range are flagged **< Conc Range**. You should report the test result as < 10 mg/dL (< 0.26 mmol/L).

Results that are above 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 1350 mg/dL (34.97 mmol/L). You may configure the system to trigger automatic reruns. Rerun results will be flagged **Autorepeat**.

Sensitivity

The ADVIA Chemistry CHOL_2 assay performance at low levels was analyzed as described in CLSI protocol EP17-A2, and the limit of blank (LoB) and limit of detection (LoD) were determined.[11]

The LoB is the highest measurement result that is likely to be observed on a blank sample. The LoB for the ADVIA Chemistry CHOL_2 assay is 2 mg/dL (0.05 mmol/L).

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 CHOL_2 assay is 10 mg/dL (0.26 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 CHOL_2 assay was evaluated according to the CLSI protocol EP5-A2.[12] Each sample was assayed 3 times per run, 2 runs per day, for at least 10 days.

Precision: Common Units

Specimen Type	N	Mean (mg/dL)	Repeatability (Within-Run) SD[a] (mg/dL)	Repeatability (Within-Run) CV[b] (%)	Between-Run SD[a] (mg/dL)	Between-Run CV[b] (%)	Between-Day SD[a] (mg/dL)	Between-Day CV[b] (%)	Within-Lab (Total) SD[a] (mg/dL)	Within-Lab (Total) CV[b] (%)
Serum Control 1	60	107	1.1	1.0	0.0	0.0	1.2	1.1	1.6	1.5
Serum Control 2	60	243	1.7	0.7	0.7	0.3	1.8	0.8	2.6	1.1
Serum Pool 1	60	176	1.5	0.9	0.9	0.5	1.7	1.0	2.5	1.4
Serum Pool 2	60	203	1.8	0.9	0.8	0.4	1.6	0.8	2.5	1.2
Serum Pool 3	60	420	3.3	0.8	0.0	0.0	4.3	1.0	5.5	1.3

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

Precision: SI Units

Specimen Type	N	Mean (mmol/L)	Repeatability (Within-Run) SD[a] (mmol/L)	Repeatability (Within-Run) CV[b] (%)	Between-Run SD[a] (mmol/L)	Between-Run CV[b] (%)	Between-Day SD[a] (mmol/L)	Between-Day CV[b] (%)	Within-Lab (Total) SD[a] (mmol/L)	Within-Lab (Total) CV[b] (%)
Serum Control 1	60	2.77	0.028	1.0	0.000	0.0	0.031	1.1	0.041	1.5
Serum Control 2	60	6.29	0.044	0.7	0.018	0.3	0.047	0.8	0.067	1.1
Serum Pool 1	60	4.56	0.039	0.9	0.023	0.5	0.044	1.0	0.065	1.4
Serum Pool 2	60	5.26	0.047	0.9	0.021	0.4	0.041	0.8	0.065	1.2
Serum Pool 3	60	10.88	0.085	0.8	0.000	0.0	0.111	1.0	0.142	1.3

[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 CHOL_2 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 CHOL_2	129	0.999	y = 0.97x + 1.0 mg/dL y = 0.97x + 0.026 mmol/L	4.9 mg/dL 0.127 mmol/L	14–669 mg/dL 0.36–17.33 mmol/L
Serum	ADVIA 1800 CHOL_2	129	1.000	y = 0.98x + 1.2 mg/dL y = 0.98x + 0.031 mmol/L	3.9 mg/dL 0.101 mmol/L	13–663 mg/dL 0.34–17.17 mmol/L
Plasma (Lithium Heparin)	ADVIA 1800 CHOL_2	65	1.000	y = 0.98x + 4.8 mg/dL y = 0.98x + 0.124 mmol/L	4.8 mg/dL 0.124 mmol/L	12–668 mg/dL 0.31–17.30 mmol/L

Specimen Type	Comparison Assay (x)	N	r	Regression Equation	Sy.x	Sample Range
Plasma[a] (Lithium Heparin) ADVIA 1650/1800	ADVIA 1650/1800 CHOL_2 - Serum	133	0.998	y = 1.02x - 2.9 mg/dL y = 1.02x - 0.08 mmol/L	9.7 mg/dL 0.25 mmol/L	77–666 mg/dL 1.99–17.25 mmol/L
Serum[b]	NCEP/CDC Reference Method	44	0.999	y = 0.99x + 1.6 mg/dL y = 0.99x + 0.04 mmol/L	2.6 mg/dL 0.07 mmol/L	111–307 mg/dL 2.87–7.95 mmol/L

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

[b] Results are from the ADVIA 2400 Chemistry system using the same reagent, with assay conditions identical to those on the ADVIA Chemistry XPT system.

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	Cholesterol Sample Concentration	Interference
Bilirubin (conjugated)	60 mg/dL (1026 µmol/L)	202 mg/dL (5.23 mmol/L)	NSI[a]
	60 mg/dL (1026 µmol/L)	240 mg/dL (6.22 mmol/L)	NSI
Bilirubin (unconjugated)	45 mg/dL (769.5 µmol/L)	201 mg/dL (5.21 mmol/L)	NSI
	60 mg/dL (1026 µmol/L)	201 mg/dL (5.21 mmol/L)	+10.0%
	45 mg/dL (769.5 µmol/L)	240 mg/dL (6.22 mmol/L)	NSI
	60 mg/dL (1026 µmol/L)	240 mg/dL (6.22 mmol/L)	+13.8%
Hemolysis (hemoglobin)	1000 mg/dL (10 g/L)	200 mg/dL (5.18 mmol/L)	NSI
	1000 mg/dL (10 g/L)	245 mg/dL (6.35 mmol/L)	NSI
Lipemia (triglycerides from Intralipid)	1000 mg/dL (11.3 mmol/L)[b]	204 mg/dL (5.28 mmol/L)	NSI
	1000 mg/dL (11.3 mmol/L)[b]	246 mg/dL (6.37 mmol/L)	NSI
Ascorbic Acid[c]	6 mg/dL (341 µmol/L)	211 mg/dL (5.46 mmol/L)	NSI

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

[b] as triolein

[c] Results are from the ADVIA 2400 Chemistry system using the same reagent, with assay conditions identical to those on the ADVIA Chemistry XPT system.

Note There is poor correlation between turbidity and triglyceride concentration in a lipemic sample.[13]

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 CHOL_2 assay is traceable to the National Cholesterol Education Program/Centers for Disease Control (NCEP/CDC) reference method, which uses reference materials from the National Institute of Standards and Technology (NIST), via patient sample correlation. See the correlation data in *Accuracy/Method Comparison* section for the relationship. Assigned values of the Siemens Chemistry Calibrator are traceable to this standardization.

Technical Assistance

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

siemens.com/healthcare

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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		

Trademarks

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