DIALAB Produktion und Vertrieb von chemisch-technischen Produkten und Laborinstrumenten Gesellschaft m.b.H.
IZ NOE-Sued, Hondastrasse, Objekt M55, 2351 Wr. Neudorf, Austria
Phone: +43 (0) 2236 660910-0, Fax: +43 (0) 2236 660910-30, e-mail: office@dialab.at

Cholesterol
CHOD-PAP

Diagnostic reagent for quantitative in vitro determination of cholesterol in human serum or plasma on photometric systems

Single reagent with ATCS*

REF	Kit Size	Configuration
D96112B	1 x 1 L	Single Reagent
D08115	4 x 250 mL	Single Reagent
D95116	5 x 100 mL	Single Reagent
D98118	5 x 50 mL	Single Reagent
D00119	5 x 25 mL	Single Reagent
D00123	5 x 10 mL	Single Reagent
D62911	10 x 50 mL	Single Reagent
D0418917	9 x 65 mL	Single Reagent
DA0814	5 x 50 mL	Single Reagent
DT1014	4 x 50 mL	Single Reagent
DK0714	5 x 50 mL	Single Reagent
DE1814	10 x 50 mL	Single Reagent
DB20312	10 x 50 mL	Single Reagent

* Advanced Turbidity Clearing System; minimizes turbidity caused by lipemia.

Additionally available:

D95114	1 x 3 mL	Cholesterol Standard	
D98485	5 x 3 mL	Calibrator	Diacal Auto
D98485SV	1 x 3 mL	Calibrator	Diacal Auto
D98481	12 x 5 mL	Control normal	Diacon N
D14481	5 x 5 mL	Control normal	Diacon N
D98481SV	1 x 5 mL	Control normal	Diacon N
D98482	12 x 5 mL	Control abnormal	Diacon P
D14482	5 x 5 mL	Control abnormal	Diacon P
D98482SV	1 x 5 mL	Control abnormal	Diacon P
D99486	3 x 3 mL	Lipid Control normal	Diacon Lipids
D99486SV	1 x 3 mL	Lipid Control normal	Diacon Lipids
D11487	3 x 3 mL	Lipid Control abnormal	Diacon Lipids High
D11487SV	1 x 3 mL	Lipid Control abnormal	Diacon Lipids High

For professional in vitro diagnostic use only.

GENERAL INFORMATION

Method	Colorimetric, enzymatic, CHOD-PAP, endpoint, increasing reaction
Shelf life	24 months from production date
Storage	2 – 8°C
Wavelength	500 nm, Hg 546 nm
Optical path	1 cm
Temperature	20 – 25 °C or 37 °C
Sample	Serum, heparin plasma or EDTA plasma

INTENDED USE

Diagnostic reagent for quantitative in vitro determination of cholesterol in human serum or plasma on photometric systems.

DIAGNOSTIC SIGNIFICANCE [1, 2]

Cholesterol is a component of cell membranes and a precursor for steroid hormones and bile acids synthesized by body cells and absorbed with food. Cholesterol is transported in plasma via lipoproteins, namely complexes between lipids and apolipoproteins. There are four classes of lipoproteins: high density lipoproteins (HDL), low density lipoproteins (LDL), very low density lipoproteins (VLDL) and chylomicrons. While LDL is involved in the cholesterol transport to the peripheral cells, HDL is responsible for the cholesterol uptake from the cells. The four different lipoprotein classes show distinct relationship to coronary atherosclerosis. LDLcholesterol (LDL-C) contributes to atherosclerotic plaque formation within the arterial intima and is strongly associated with coronary heart disease (CHD) and related mortality. Even with total cholesterol within the normal range an increased concentration of LDL-C indicates high risk. HDL-C has a protective effect impeding plaque formation and shows an inverse relationship to CHD prevalence. In fact, low HDL-C values constitute an independent risk factor. The determination of the individual total cholesterol (TC) level is used for screening purposes while for a better risk assessment it is necessary to measure additionally HDL-C and LDL-C.
In the last few years several controlled clinical trials using diet, life style changes and / or different drugs (especially HMG CoA reductase inhibitors [statins]) have demonstrated that lowering total cholesterol and LDL-C levels reduce drastically CHD risk [2].

TEST PRINCIPLE

Determination of cholesterol after enzymatic hydrolysis and oxidation [3,4]. The colorimetric indicator is quinoneimine which is generated from 4-aminoantipyrine and phenol by hydrogen peroxide under the catalytic action of peroxidase (Trinder's reaction) [3].

$$\text{Cholesterol ester} + H_2O \xrightarrow{CHE} \text{Cholesterol} + \text{fatty acid}$$

$$\text{Cholesterol} + O_2 \xrightarrow{CHO} \text{Cholesterol-3-one} + H_2O_2$$

$$2\ H_2O_2 + \text{Phenol} + 4\ \text{Aminoantipyrine} \xrightarrow{POD} \text{Quinonimine} + 4\ H_2O$$

The intensity of the pink/red colour is proportional to the Cholesterol concentration in the sample.

REAGENT COMPOSITION

COMPONENTS	CONCENTRATION	
Good´s buffer, pH 6.7	50	mmol/L
Phenol	5	mmol/L
4-Aminoantipyrine	0.3	mmol/L
Cholesterol esterase (CHE)	≥ 200	U/L
Cholesterol oxidase (CHO)	≥ 50	U/L
Peroxidase (POD)	≥ 3	kU/L

MATERIAL REQUIRED BUT NOT PROVIDED

- NaCl solution (9 g/L).
- Clinical chemistry analyser.

REAGENT PREPARATION

The reagent provided is ready to use.

STORAGE AND STABILITY

Conditions:	Protect from light Close immediately after use Avoid contamination Do not freeze the reagent.
Storage:	at 2 – 8 °C
Stability:	up to the indicated expiration date

Note: The measurement is not influenced by occasionally occurring colour changes, as long as the absorbance of the reagent is < 0.3 at 546 nm.

WARNINGS AND PRECAUTIONS

1. The reagent contains sodium azide (0.95 g/L) as preservative. Do not swallow! Avoid contact with skin and mucous membranes.
2. Standard: Warning.

H317: May cause an allergic skin reaction.
H319: Causes serious eye irritation.
P264: Wash hands and face thoroughly after handling.
P280: Wear protective gloves/protective clothing/eye protection/face protection.
P302+P352: If on skin: Wash with plenty of soap and water.
P337+P313: If eye irritation persists: Get medical advice/attention.
Special labelling: Contains 2-Chloracetamide and Isotridecanol, ethoxylated.

3. In very rare cases, samples of patients with gammopathy might give falsified results [8].
4. N-acetylcysteine (NAC), acetaminophen and metamizole medication leads to falsely low results in patient samples.
5. Please refer to the safety data sheet and take the necessary precautions for the use of laboratory reagents.
6. For diagnostic purposes, the results should always be assessed with the patient's medical history, clinical examinations and other findings.
7. For professional use only!

SPECIMEN COLLECTION AND STORAGE

Stability [6]:	at 20 – 25 °C	7 days
	at 4 – 8 °C	7 days
	at - 20 °C	3 months

Only freeze once!
Discard contaminated specimens.

STANDARD

(not included in the kit; has to be ordered separately)

Concentration	200 mg/dL (5.2 mmol/L)
Storage:	2 – 8 °C
Stability:	up to the indicated expiration date

Close immediately after use! Avoid contamination!
Protect from light.

TEST PROCEDURE

Bring reagents and samples to room temperature.

Pipette into test tubes	Blank	Std./Cal.	Sample
Reagent	1000 µL	1000 µL	1000 µL
Sample	-	-	10 µL
Standard/Calibrator	-	10 µL	-
Dist water	10 µL	-	-
Mix. Incubate 10 minutes at 37 °C or 20 minutes at 20 – 25 °C. Read absorbance of sample and Std./Cal. within 60 minutes against the reagent blank.			

Automation

Special adaptations for automated analysers can be made on request.

INTERPRETATION OF RESULTS

Calculation

With standard or calibrator

$$\text{Cholesterol [mg/dL]} = \frac{\text{A Sample}}{\text{A Std/Cal}} \times \text{conc. Std/Cal [mg/dL]}$$

Unit Conversion

Cholesterol [mg/dL] x 0.02586 = Cholesterol [mmol/L]

QUALITY CONTROL AND CALIBRATION

All control sera with Cholesterol values determined by this method can be used.
We recommend the Dialab lipid control sera **Diacon Lipids** and **Diacon Lipids High** and the Dialab multi control sera **Diacon N** (with values in the normal range) and **Diacon P** (with values in the pathological range).

Each laboratory should establish corrective action in case of deviations in control recovery.

Calibration
The assay requires the use of a Cholesterol standard or calibrator.
We recommend the Dialab **Cholesterol Standard** or the multi calibration serum **Diacal Auto**.

PERFORMANCE CHARACTERISTICS

LINEARITY, MEASURING RANGE
The test has been developed to determine cholesterol concentrations within a measuring range from 3 – 750 mg/dL (0.08 – 19.4 mmol/L). If values exceed this range, samples should be diluted 1 + 4 with NaCl solution (9 g/L) and the result multiplied by 5.

SENSITIVITY/LIMIT OF DETECTION
The lower limit of detection is 3 mg/dL (0.08 mmol/L).

PRECISION (at 37 °C)

Intra-assay n = 20	Mean [mg/dL]	SD [mg/dL]	CV [%]
Sample 1	108	1.76	1.62
Sample 2	236	1.45	0.61
Sample 3	254	1.57	0.62

Inter-assay n = 20	Mean [mg/dL]	SD [mg/dL]	CV [%]
Sample 1	104	1.19	1.14
Sample 2	211	2.57	1.22
Sample 3	245	2.28	0.93

SPECIFICITY/INTERFERENCES

no interference up to:

Ascorbic acid	5 mg/dL
Bilirubin	20 mg/dL
Hemoglobin	200 mg/dL
Triglycerides	2000 mg/dL

For further information on interfering substances refer to Young DS [7].

METHOD COMPARISON

A comparison between Dialab Cholesterol (y) and a commercially available test (x) using 78 samples gave following results: y = 1.00 x – 2.50 mg/dL; r = 0.995.

TRACEABILITY

The assigned values of Diacal Auto and Cholesterol Standard have been made traceable to the reference method gas chromatography – isotope dilution mass spectrometry (CG-IDMS).

EXPECTED VALUES [5]*

Desirable	≤ 200 mg/dL (5.2 mmol/L)
Borderline high risk	200 – 240 mg/dL (5.2 – 6.2 mmol/L)
High risk	> 240 mg/dL (> 6.2 mmol/L)

* Each laboratory should check if the reference ranges are transferable to its own patient population and determine own reference ranges if necessary.

Clinical Interpretation
The European Task Force on Coronary Prevention recommends to lower Total Cholesterol concentration to less than 190 mg/dL (5.0 mmol/L) and LDL-cholesterol to less than 115 mg/dL (3.0 mmol/L) [2].

LIMITATIONS

- Eventual Cholesterol, CHOD-PAP carry-over to reagents Magnesium (Xylidyl blue), Iron (Ferene), Lipase (Enzymatic, colorimetric) and Protein Total in Urine/CSF (Pyrogallol red). The actual carry-over depends on the analyser.

WASTE MANAGEMENT

Please refer to local legal requirements.

LITERATURE

1. Rifai N, Bachorik PS, Albers JJ. Lipids, lipoproteins and apolipoproteins. In: Burtis CA, Ashwood ER, editors. Tietz Textbook of Clinical Chemistry. 3rd ed. Philadelphia: W.B. Saunders Company; 1999. p 809-61.
2. Recommendation of the Second Joint Task Force of European and other Societies on Coronary Prevention. Prevention of coronary heart disease in clinical practice. Eur Heart J 1998; 19: 1434-503.
3. Artiss JD, Zak B. Measurement of cholesterol concentration. In: Rifai N, Warnick GR, Dominiczak MH, eds. Handbook of lipoprotein testing. Washington: AACC Press, 1997: p. 99-114.
4. Deeg R, Ziegenhorn J. Kinetic enzymatic method for automated determination of total cholesterol in serum. Clin Chem 1983; 29: 1798-802.
5. Schaefer EK, McNamara J. Overview of the diagnosis and treatment of lipid disorders. In: Rifai N, Warnick GR, Dominiczak MH, eds. Handbook of lipoprotein testing. Washington: AACC press, 1997: p. 25-48.
6. Guder WG, Zawta B et al. The quality of Diagnostic Samples. 1st ed. Darmstadt: GIT Verlag; 2001. p. 22-3.
7. Young DS. Effects of Drugs on Clinical laboratory Tests. 5th ed. Volume 1 and 2. Washington, DC: The American Association for Clinical Chemistry Press 2000.
8. Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. ClinChemLabMed 2007; 45(9): 1240-1243.

CE IVD