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CUSTOMISED SOLUTIONS FOR YOUR LABORATORY

Cholesterol PAP – Instructions for use

CE IVD

R1: 4x 17,5 mL **R3330000014** REF

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

TEST PARAMETERS

Method:	Colorimetric, enzymatic, CHOD-PAP, endpoint, increasing reaction
Wavelength	500 nm, Hg 546 nm
Temperature:	20 – 25 °C or 37 °C
Sample:	Serum, heparin plasma or EDTA plasma
Linearity:	up to 750 mg/dL (19.4 mmol/L)
Sensitivity:	The lower limit of detection is 3 mg/dL (0.08 mmol/L)

SUMMARY [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. 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-Cholesterol and LDL-Cholesterol.

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-Cholesterol 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 peroxidise (Trinder's reaction) [3].

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

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

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

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

REAGENT COMPOSITION

COMPONENTS	CONCENTRATIONS
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

REAGENT PREPARATION

The reagent is ready to use.

REAGENT STABILITY AND STORAGE

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.

SAMPLE STABILITY AND STORAGE [6]

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

Discard contaminated specimens! Freeze only once!

MATERIALS REQUIRED BUT NOT PROVIDED

NaCl solution (9 g/L)
General laboratory equipment

STANDARD

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

Concentration:	200 mg/dL (5.20 mmol/L)
Storage:	2 – 25 °C
Stability:	up to the indicated expiration date

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

MANUAL 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.			

CALCULATION

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

UNIT CONVERSION

mg/dL x 0.02586 = mmol/L

REFERENCE RANGE [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)

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* 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].

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 this 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.

CALIBRATION

The assay requires the use of a Cholesterol standard or calibrator. We recommend our Multicalibrator for which the assigned values have been made traceable to the reference method gas chromatography – isotope dilution mass spectrometry (CG-IDMS).

QUALITY CONTROL

All control sera with Cholesterol values determined by this method can be used. We recommend our serum controls Normal control serum (control serum with values in the normal range) and Pathological control serum (control serum with values in the abnormal range).

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

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.
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!

WASTE MANAGEMENT

Please refer to local legal requirements.

REFERENCES

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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.
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Symbols on labels and packaging

IVD	=	In vitro diagnostic medical device
REF	=	Catalog Number
LOT	=	Lot Number
	=	Manufacturer
	=	Expiration date
	=	Temperature limitation
	=	Instruction for use

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