CHOLESTEROL CHOD-PAP

Diagnostic reagent for the in-vitro quantitative determination of cholesterol in human serum on both manual and automated systems.

REF:V/CH02.025	50 test	REF:V/CH02.125	250 test
REF:V/CH04.025	100 test	REF:V/CH04.125	500 test
REF:V/CH02.050	100 test	REF:V/CH04.250	1000 test
REF:V/CH02.100	200 test		

CLINICAL SIGNIFICANCE

Measurement of serum cholesterol levels is important as an indicator of liver function, intestinal absorption, biliary function and in the diagnosis and classification of hyperlipoproteinemias. Elevated cholesterol levels may occur with hypothyroidism, diabetes and nephrotic syndrome. Elevated serum cholesterol levels correlate well with the incidence of coronary artery diseases. Stress, age gender, hormonal balance and pregnancy affect normal cholesterol levels. Depressed levels are associated with hyperthyroidism and severe liver diseases.

METHOD PRINCIPLE

CHOD-PAP-enzymatic colorimetric method.

The series of the reactions involved in the assay system is as follows:

1. Cholesterol esters are enzymatically hydrolyzed by cholesterol esterase (CHE) to cholesterol and free fatty acids.

$$\text{Cholesterol esters} \xrightarrow{\text{CHE}} \text{Cholesterol} + \text{Fatty acids}$$

2. Free cholesterol, including that originally present, is then oxidized by cholesterol oxidase (CHOD) to cholest-4-en-3-one and hydrogen peroxide.

$$\text{Cholesterol} + O_2 \xrightarrow{\text{CHOD}} \text{Cholest-4-en-3-one} + H_2O_2$$

3. The hydrogen peroxide combines with phenol and 4-amino-antipyrine (4AAP) in the presence of peroxidase (POD) to form a chromophore (quinoneimine dye) which may be quantitated at 500 – 550 nm.
   For bichromatic analyzers the blank wavelength should be set to 600 or 650 nm.

$$2H_2O_2 + \text{Phenol} + 4\text{AAP} \xrightarrow{\text{POD}} \text{Quinoneimine Dye} + 4H_2O$$

REAGENT COMPOSITION

R 1: Standard	- 200 mg/dl - 5.17 mmol/L
R2: Reagent	
- Pipes Buffer pH 7.04	- 50 mmol/L
- Phenol	- 30 mmol/L
- Sodium cholate	- 5.0 mmol/L
- Cholesterol esterase	- >250 U/L
- Cholesterol oxidase	- >500 U/L
- Peroxidase	- >2.0 KU/L
- 4-amino-antipyrine	- 1.0 mmol/L
- Sodium Azide	- 8.0 mmol/L

PRECAUTIONS AND WARNINGS

Reagent to be handled by entitled and professionally educated person. Do not ingest or inhale as reagent contains sodium azide which is classified as dangerous substance for environment.

Good Laboratories practices using appropriate precautions should be followed in:

- Wearing personnel protective equipment (overall, gloves, glasses,).
- Do not pipette by mouth.
- In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.
- Respect country requirement for waste disposal.
  S56: dispose of this material and its container at hazardous or special waste collection point.
  S57: use appropriate container to avoid environmental contamination.
  S61: avoid release in environment.

For further information, refer to the **Lab.Vie**. Cholesterol reagent material safety data sheet.

REAGENT PREPARATION, STORAGE AND STABILITY

Lab.Vie. Cholesterol reagents are supplied ready-to-use and stable up to the expiry date labeled on the bottles when properly stored refrigerated at 2–8°C. Once opened, the opened vial is stable for 4 months at 2–8°C.

Deterioration
The **Lab.Vie**. Cholesterol reagent is normally clear or pale pink. Do not use Cholesterol reagent if it is turbid or if the absorbance is greater than 0.15 at 546 nm.

SPECIMEN COLLECTION AND PRESERVATION

It is recommended that prior to sample collection, patients should be following their usual diet and be in their usual state of health. Patients who are actually ill, losing weight, pregnant or have had a myocardial infarction in the previous 3 months should be rescheduled. Both fasting and non-fasting samples can be used. Non hemolyzed serum or heparinized plasma can be stored at 4°C up to 7 days prior to analysis, 5-7 days at 20°- 25°C, stable for 3 months at -20°C, and at -70°C for several months.

SYSTEM PARAMETERS

Wavelength	546 nm (492 – 550 nm)
Optical path	1 cm
Assay type	End-point
Direction	Increase
Sample Reagent Ratio e.g: Reagent volume Sample volume	1:100 1 ml 10 µl
Temperature	15– 25°C or 37 °C
Incubation time	10 min. at 15–25°C or 5 min. at 37°C
Zero adjustment	Reagent Blank
Reagent Blank Limits	Low 0.00 AU High 0.15 AU
Sensitivity	5 mg/dL (0.27 mmol/L)
Linearity	750 mg/dL (19.5 mmol/L)

EQUIPMENT REQUIRED NOT PROVIDED

- Sterile Syringe
- Analytical tubes and automatic pipet
- Centrifuge and spectrophotometer

ASSAY PROCEDURE

	Blank	Standard	Specimen
Reagent (R)	1.0 ml	1.0 ml	1.0 ml
Standard		10 µl	
Specimen			10 µl

Mix and incubate for 5 minutes at 37°C or 10 minutes at 15-25°C. Measure absorbance of specimen "A" and standard "A" against reagent blank within 30 minutes.

CALCULATION

Cholesterol concentration (mg/dl) = $\frac{\text{(A specimen)} \times 200}{\text{(A standard)}}$

QUALITY CONTROL

To ensure adequate quality control, it is recommended that normal and abnormal commercial control serum of known concentrations included in each run. If control values are found outside the defined range, check the instrument calibration, and reagent for problems. If control still out of range please contact **Lab.Vie**. technical support.

PERFORMANCE CHARACTERISTICS

Precision	Within run (Repeatability)		Run to run (Reproducibility)	
	Normal level	High level	Normal level	High level
n	20	20	20	20
Mean mg/dl	149.8	252	157	259
SD.	1.69	1.91	1.77	2.12
CV. %	1.13	0.76	1.23	0.97

The results of the performance characteristics depend on the analyzer used.

Accuracy (Methods Comparison)
Result obtained from **Lab.Vie**.Cholesterol reagent compared with commercial reagent of the same methodology performed on 20 human sera give a correlation of 0.988.

Sensitivity
When run as recommended, the minimum detection limit of the assay is 5 mg/dL (0.13 mmol/L).

Linearity
The reaction is linear up to Cholesterol concentration of 750 mg/dl; specimens showing higher concentration should be diluted 1+1 using physiological saline and repeat the assay (result×2).

INTERFERING SUBSTANCES (3)

Haemolysis
No significant interference from haemoglobin up to 500 mg/dl.
Icterus
No significant interference from free bilirubin up to levels of 15 mg/dl (260 mmol/L) and conjugated bilirubin up to levels of 7 mg/dl (116 mmol/L).
lipemia
No significant interference up to 1.7 AU.
Others
Physiological ascorbic acid concentration does not interfere with the test. Ascorbic Acid levels higher than 425 mmol/l (7.5 mg/dl) decrease the apparent total cholesterol concentration significantly.

EXPECTED VALUES

Serum	mg/dl	[mmol/L]
Desirable	<200	<5.2
Borderline high	200-239	5.2-6.2
High	>240	>6.2

DYNAMIC RANGE

5 - 750 mg/dl (0.13 – 19.5 mmol/L).

REFERENCES

1. Ellefson RD and Caraway WT: Fundamentals of clinical chemistry. Ed Tietz NW 1976; p506.
2. Flegg HM: Ann Clin Biochem 1963; 10: 79.
3. NCEP expert panel, Arch Intern Med 1988; 148: 36–69
4. Richmond. N., Clin. Chem. 1973; 19: 1350-1356.
5. Roeschlau,P., Bernt. E. and Gruber. W.J., Clin. Chem Clin. Biochem. 1974; 12:403.
6. Trinder, P, Ann. Clin. Biochem. 1969; 6: 24.
7. Young DS.et al. Clin Chem. 1975; 21.

SYMBOLS IN PRODUCT LABELLING

IVD	For in-vitro diagnostic use	Σ	Number of <n> test in the pack
LOT	Batch Code/Lot number		Caution
REF	Catalogue Number		Do not use if package is damaged
	Temperature Limitation		Consult Instruction for use
	Expiration Date		
	Manufactured by		

Ismailia – Free zone, Ismailia – Egypt
Post code-41511
E-mail : admin@labvielab.com
Website: www.labvielab.com