



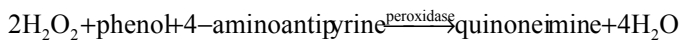
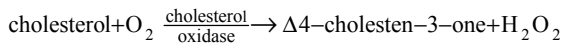
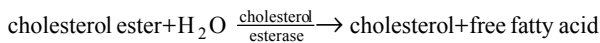
# CHOLESTEROL

## Enzymatic Colorimetric Method (CHOD-PAP)

Cat.No. 101-0237	Size 5 x 20 ml
Cat.No. 101-0012	Size 4 x 50 ml
Cat.No. 101-0267	Size 12 x 50 ml
Cat.No. 101-0050	Size 6 x 100 ml
Cat.No. 101-0051	Size 4 x 250 ml

### PRINCIPLE:

Cholesterol esterase catalyse the hydrolysis of cholesterol esters into free cholesterol and fatty acids. The free cholesterol is then oxidized to 4-cholesten-3-one and hydrogen peroxide in the presence of cholesterol oxidase. Phenol and 4-aminoantipyrine then combine with the hydrogen peroxide in the presence of peroxidase to produce red quinoneimine. The intensity of the color thus produced is directly proportional to the total cholesterol concentration of the sample.



### SAMPLE:

Serum or heparinized or EDTA - plasma.  
Cholesterol in serum is stable for 7 days at +15 °C to +25 °C and six months frozen ( - 20 °C ).

### REAGENTS:

- Buffer  
Pipes buffer, pH 6.9 90 mmol/L  
Phenol 26 mmol/L
- Enzyme/substrate reagent  
Cholesterolesterase 300 U/L  
Cholesteroxidase 200 U/L  
Peroxidase 1250 U/L  
4-aminoantipyrine 0.4 mmol/L
- Cholesterol standard  
Standard concentration see on the vial label

Store at +2 °C to +8 °C

### PREPARATION OF REAGENTS

(Working reagent):

Dissolve contents of reagent Enzyme/substrate (bottle 2) with the corresponding volume of buffer (bottle 1). The working solution is stable for 2 months at +2 °C to +8 °C or 14 days at +15 °C to +25 °C.

Standard is ready for use.

### PROCEDURE:

Wavelength:	505 nm (500 - 550 nm) or Hg 546 nm
Cuvette:	1 cm light path
Temperature:	room temperature or 37 °C
Color stability:	60 min.
Zero:	reagent blank

Pipette into test tubes:	Reagent blank	Standard	Sample
Standard	-	10 µl	-
Sample	-	-	10 µl
Working reagent	1000 µl	1000 µl	1000 µl
Mix, incubate 10 min. at room temperature or 5 min. at 37 °C. Measure absorbance of sample and of standard against the reagent blank within 60 min.			

### CALCULATION:

$$\frac{A_{\text{sample}}}{A_{\text{standard}}} \times \text{stand.conc.} = \text{mmol/L cholesterol}$$

### EXPECTED VALUES:

Normal values:	up to 5.7 mmol/L (220 mg/dl)
Suspect:	5.7 - 6.7 mmol/L (220 - 260 mg/dl)
Elevated:	> 6.7 mmol/L (260 mg/dl)

### LINEARITY:

up to 20.8 mmol/L (804 mg/dl)

### QUALITY CONTROL:

CONTRO-N	20 x 5 ml	Cat. No. 101-0083
CONTRO-P	20 x 5 ml	Cat. No. 101-0084

### NOTE:

- Sample with cholesterol concentration > 20.8 mmol/L has to be diluted 1:3 with physiological solution ( result x 3 ).
- Haemoglobin up to 2 g/L, bilirubin up to 85 µmol/L, triglycerides up to 11 mmol/L and proteins up to 100 g/L do not interfere with test.
- Ascorbic acid's concentration ≥ 50 mg/L, methyl dopa ≥ 50 mg/L, 4-methylaminoantipyrine ≥ 120 mg/L decrease results.
- Working solution must not get in touch with skin and mucous membranes (the reagents contain phenol).

### REFERENCES:

- Richmond, W., Clin.Chem. 19,1350 - 1356 (1973).
- Thomas, L., Labor und Diagnose, 2 Aufl. (1984).
- Flegg, H.M., Ann. Clin. Biochem 10, 79 - 84 (1973).