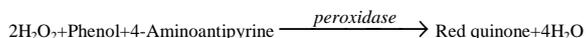
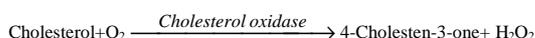
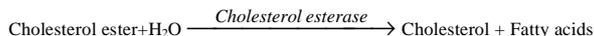


**Reagent kit for the quantitative determination of total cholesterol concentration in serum. Enzymatic colorimetric method (PAP).**

The biosynthesis of Cholesterol predominantly takes place in the liver and in intestinal mucosa, but almost all cells synthesize it. It is a constituent of many membranes, it is also essential in the synthesis of bile acids and steroid hormones. It circulates in blood as cholesterol ester bound to beta lipoproteins. The measurement of the level of Cholesterol as well as Triglycerides and Lipoproteins is important in examining the metabolism of lipids. Changes in the level of Cholesterol mainly reflect disorders of liver function. Cholesterol level is increased in obstructive jaundice, diabetes mellitus and hypothyroidism. The level is decreased in some cases of hyperthyroidism and certain forms of anaemia. Identification of the different density fractions (HDL, LDL, VLDL) as well as total Cholesterol plays a role in the diagnosis.

**Principle**

The Cholesterol esters of the sample are hydrolysed by Cholesterol esterase (ChEH). 4-Cholesten-3-one and H<sub>2</sub>O<sub>2</sub> are then formed from the released free Cholesterol by Cholesterol oxidase (ChOD). A measurable Red quinonimine derivative which absorbance light at 505 nm is formed from Hydrogenperoxide (H<sub>2</sub>O<sub>2</sub>) and 4-Aminoantipyrine in the presence of Phenol and peroxidase (POD).



**Reference values**

**Serum cholesterol:** 2.8-5.2 mmol/l (109-202 mg/dl)

It is recommended that each laboratory should assign its own normal range.

**Reagents**

**1. Reagent (R1)**

Pipes buffer, pH=6.90	50 mmol/l
Phenol	24 mmol/l
Sodium cholate	0.5 mmol/l
4-Aminoantipyrine	0.5 mmol/l
Cholesterol esterase	180 U/l
Cholesterol oxidase	200 U/l
Peroxidase	1000 U/l

**2. Cholesterol standard**

Ready for use. For details please check the insert.

Available only in Cat. No.: 47062S and 47061S

**Precautions**

Discard cloudy reagent. Avoid contamination by using clean laboratory materials (pipettes, plastic vials...). The reagents contain 0.1 % sodium azide. To avoid the possible build-up of azide compounds, flush waste-pipes with water after the disposal of undiluted reagent.

**PROCEDURE**

**Preparation and stability of working reagents**

The reagent is ready for use.

If the absorbance of working reagent is higher than 0.28 at 492 nm the reagent can not be used.

**Samples**

Serum free of haemolysis.

**Assay conditions**

Wavelength:	505 (480-520) nm
Temperature:	37°C
Cuvette:	1 cm light path
Read against:	reagent blank
Method:	endpoint (increasing)

**Pipette into cuvette**

	Blank	Standard	Sample
Reagent	1 ml	1 ml	1 ml
Distilled water	10µl		
Standard		10µl	
Sample			10µl

Mix and read the absorbance (A) after a 5-minute incubation.

**Calibration:** (37°C, Cholesterol oxidase method)

- S1: Distilled water
- S2: Cholesterol standard Cat. No.: 50611N or Roche C.F.A.S. (Calibrator for automated system)
- Randox Calibration Serum Level I or
- Randox Calibration Serum Level II

**Calibration frequency:**

- Two point calibration is recommended
- after reagent lot change,
- as required following quality control procedures.

**Calculation using calibration**

$$\frac{A_{\text{sample}}}{A_{\text{standard}}} \times C_{\text{standard}} = C_{\text{sample}}$$

A = Absorbance,  
C = Concentration

**Quality control**

A quality control program is recommended for all clinical laboratories. The analysis of control material in both the normal and abnormal ranges with each assay is recommended for monitoring the performance of the procedure. Each laboratory should establish corrective measures to be taken if values fall outside the limits.

**PERFORMANCES DATA**

The following data were obtained using the Olympus 400 analyzer (37°C).

**Linearity**

Up to 20.0 mmol/l (773 mg/dl).

**Sensitivity**

It is recommended that each laboratory establishes its own range of sensitivity as this is limited by the sensitivity of the spectrophotometer used. Under manual conditions however, a change of 0.001 Abs is equivalent to 0.015 mmol/l (0.58 mg/dl) Cholesterol concentration at 492 nm.

**Precision**

	Reproducibility		
	Average concentration (mmol/l)	SD	CV%
Sample I	2.5	0.044	1.79
Sample II	5.4	0.093	1.72

	Repeatability		
	Average concentration (mmol/l)	SD	CV%
Sample I	3.6	0.034	1.02
Sample II	11.8	0.094	0.80

**Correlation**

Comparative studies were done to compare our reagent with another commercial Cholesterol PAP reagent.

The results from these studies are detailed below.

Correlation coefficient: r = 0.9994

Linear regression: y (mmol/l) = 1.006x - 0.105

(x = other commercial reagent, y = own reagent).

**Specificity**

Bilirubin 855 µmol/l (50 mg/dl), lipid 450 mg/dl, glucose 55.5 mmol/l (1000 mg/dl) and ascorbic acid 0.6 mmol/l (10 mg/dl) don't interfere with the assay up to the given levels.

**NOTE**

Do not use reagents after the expiry date stated on each reagent container label. Do not use products, test solutions and reagents described above for any purpose other than described herein.

**For in vitro diagnostic use only.**

**The following symbols are used on labels**

 For in vitro diagnostic use

 Use by (last day of the month)

 Temperature limitation

 Batch Code

 Code

**Bibliography**

Allain C.C and al., Clin.Chem.,20, (1974), 470.