Intended Use
For the quantitative determination of Cholesterol in serum.

Clinical Significance
Determinations of Cholesterol are primarily used for diagnosing atherosclerosis and heart disease, liver disease and renal disease, as well as for monitoring the causes and treatment. (1)

Method History
Enzymatic Cholesterol methods were introduced in the early nineteen seventies when Cholesterol Oxidase (EC1.1.3.6) and Cholesterol Esterase (EC 3.1.1.13) became available. Allain, et al, (1) and Roeschlau, et al (2) described the use of a single enzymatic reagent for the assay of Cholesterol in serum. Later, L.P.Leon, et al (3) automated these methods for use in continuous flow systems. Catachem's enzymatic Cholesterol method described below uses a combination of microbial Cholesterol Oxidase, pancreatic Cholesterol Esterase of animal source and a colorimetric end-point reaction based upon the work of Trinder. (4)

Method Principle
Cholesterol esters present in the serum sample are hydrolyzed into free Cholesterol and fatty acids by pancreatic Cholesterol esterase (CE). The free Cholesterol formed and in the presence of oxygen, is then oxidized to cholest-4-en-3-one by Cholesterol Oxidase (CO) with concomitant production of hydrogen peroxide. The hydrogen peroxide produced is quantitatively determined by coupling 4-aminoantipyrine with p-hydroxybenzoic acid (5) where a quinoneimine dye with maximum absorption at 500nm is produced. The intensity of the dye color thus produced is directly proportional to the concentration of the total Cholesterol in the serum sample. The reaction scheme illustrates the reactions that occur in this method.

$$\text{Cholesterol esters} \xrightarrow{\text{CE}} \text{Cholesterol} + \text{fatty acids}$$

$$\text{Cholesterol} + 02 \xrightarrow{\text{C0}} \text{Cholest-4-en-3-one} + H202$$

$$2H202 + \text{4-aminoantipyrine} + \text{p-hydroxybenzoic acid} \xrightarrow{\text{peroxidase}} \text{quinoneimine dye} + 4H20$$

Reagent Content
Active ingredients in the reagents will be approximately as follows:

Cholesterol Reagent
Each liter contains:

Peroxidase	≥1600 U
Cholesterol Oxidase (microbial)	≥150 U
Cholesterol Esterase (pancreatic)	≥125 U
4-aminoantipyrine	0.6 mM
p-hydroxybenzoic acid	18.7 mM
Buffer and non reactive ingredients	

Precaution
Avoid contact of the reagent with skin and eyes. Should contact occur, wash affected area with plenty of cold water. **DO NOT PIPETTE REAGENTS BY MOUTH.**

Preparation Of Working Reagent
Catachem Cholesterol reagent is packaged in ready-to-use form. No preparation is required.

Storage And Stability
Store the Cholesterol reagent at 2-8°C. When stored as directed, the reagent is stable until the expiration date stated on the label. When stored as directed, the Working Cholesterol Reagent is stable for 12 months at 2-8°C and 12 weeks at room temperature.

Specimen Collection And Preparation
Fresh unhemolyzed serum is the specimen of choice. No preservatives are necessary. Cholesterol in human serum samples is stable for one week at 2-8 °C and for six months at –20 °C.

Interfering Substances
A number of substances have been reported to affect the accuracy of cholesterol methods using the Cholesterol Oxidase peroxidase procedures. (6) A summary of the influence of drugs on clinical laboratory procedures may be found by consulting D.S. Young, et al. (7)

Expected Values
Based on the recommendations of the NIH Conference to review the scientific significance of Cholesterol values on human subjects (8) the following risk cutoff levels for the American population should be observed:

Ideal normal serum Cholesterol	≤200 mg/dL
Borderline serum Cholesterol	200-239 mg/dL
High serum Cholesterol	≥240 mg/dL

These values are suggested guidelines. It is recommended that each laboratory establish the normal range for the area in which it is located.

Directions For Use
Catachem's Cholesterol method requires one single reagent. The reagent is ready for use and requires no preparation. The Working Reagent is stable twelve months aboard the analyzer at 2-8˚C and capped while not in use.

Procedure
Important: Read the entire procedure instructions before proceeding with assay.

Materials Required But Not Provided

Spectrophotometer	
Match cuvettes	1 cm light path
Timer	to time incubation time
Pipette	1.0 ml for reagent
Pipette	0.010 ml for sample

Materials Provided
Cholesterol Reagent

Analytical Parameters

Wavelength	550nm
Temperature	37˚C
Pathlength	1 cm
Reaction Mode	endpoint
Reaction Time	5 min
Reagent Volume	1.0 ml
Sample Volume	0.01 ml (10uL)
Total Volume	1.01 ml
Sample-to-reagent Ratio	1:100

Assay Procedure
1. Pipette 1.0 ml of Cholesterol reagent into each of three cuvettes marked "calibrator", "sample", and "blank".
2. Pipette 0.01 ml (10 uL) of calibrator or sample into their respective cuvettes. Mix all cuvettes well.
3. Incubate all cuvettes for 5 minutes at 37˚C.
4. Set spectrophotometer wavelength at 550nm and zero the instrument with the cuvette marked "blank".
5. Read the "calibrator" and "sample" absorbencies.
6. Calculate the cholesterol concentration (mg/dL) in the sample(s), as shown in results and calculations.

Results And Calculations

$$\text{Cholesterol (mg/dL)} = \frac{\text{sample abs}}{\text{calibrator abs}} \times \text{calibrator (mg/dL)}$$

Example:

Sample absorbance	=	0.300
Calibrator absorbance	=	0.250
Calibrator (mg/dL)	=	200

$$\text{Cholesterol (md/dL)} = \frac{0.300}{0.250} \times 200 = 240 \text{ mg/dL}$$

Quality Control
To monitor the performance of the Working Reagent and the procedure used we recommend the regular use of a normal and abnormal control serum.

Method Performance Characteristics
Sensitivity: 0.0010-0.0020 absorbance units per mg/dL.
Linear Range: 0-600 mg/dL.
Precision: Within-run and day-to-day precision is summarized below.

Cholesterol Precision Study

Cholest.	Within-Run		Total Precision	
Mean	SD	CV	SD	CV
mg/dL	mg/dL	%	Mg/dL	%
56	0.71	1.2	0.71	1.3
261	1.95	0.75	6.3	2.4
456	2.97	0.65	3.0	0.70

Correlation
A comparison of this method using an automated analyzer and a reference method based on the CO and CE reaction resulted in the following regression statistics:

N	=	155
Y	=	1.003x + 4.3
r	=	0.998
Sy.x	=	5.7

References

1. Allain CC, Poon LS, Chan CSG, Richmond W, Fu PC. Clin Chem 20, 470 (1974).
2. Roeschlau P, Bernt E. Gruber W.Clin ChemClin Biochem 12, 226 (1974).
3. Leon LP, Chu DK, Stasiw RO, Snyder: LR. Advances in Automated Analysis. Technicon's International congress, 152-156 (1976).
4. Trinder P. Ann Clin Biochem. 6, 24 (1969).
5. Bardelli F, Gianninin G, Meiattini F, Prencipe L, Tarli P. Clin Chem 24, No. 12 (1978).
6. Pesce MA, Bodourian SH. Clin Chem 23, 757-760 (1977).
7. Young DS, Pestaner LC, Gibberman V. Clin Chem 21, No. 5 (1975).
8. Report of the National Cholesterol Education Program Expert Panel on Detection,\ Evaluation and Treatment of High Blood Cholesterol in Adults. Arch of Int Med. Vol 148, No. 1 pp 36-69. Jan. 1988.

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