CHOLESTEROL

(LIQUID)

4 x 60 ml

RE – ORDER CHL1100

INTENDED USE:

This reagent is intended for the rapid quantitative in vitro measurement of total serum or plasma cholesterol concentration, utilizing manual or automated analyzers.

The determination of serum cholesterol is one of the important tools in the diagnosis and classification of lipemias. Other conditions, such as hepatic and thyroid diseases, influence cholesterol levels.

TEST SUMMARY:

The elegant and simple enzymatic method of assay has replaced older methodologies. The method employed in this reagent is based on the work of Allain et al.[1].

In this method, cholesterol esters are hydrolyzed by cholesterol esterase to cholesterol and fatty acids:

Cholesterol

Cholesterol esters ——————→ Cholesterol + fatty acids

esterase

Cholesterol is oxidized by cholesterol oxidase to Δ4-Cholestenone with the simultaneous production of hydrogen peroxide:

Cholesterol

Cholesterol + O_2 ——————→ Δ4-Cholestenone + $4H_2O_2$

Oxidase

In the presence of peroxidase, hydrogen peroxide oxidizes phenol and 4-aminoantipyrine to give a quinoneimine dye colored in red:

$2H_2O_2$ + 4-aminoantipyrine + phenol

Peroxidase

——————→ Quinoneimine dye + $4H_2O$

The intensity of the color produced is proportional to the concentration of cholesterol in the sample.

REAGENT DESCRIPTION

Cholesterol Rapid Liquid Reagent

Reactive ingredients:	
4-Aminoantipyrine	0.6 mmol/L
Phenol	10 mmol/L
Cholesterol Oxidase	≥ 300 U/L
Cholesterol Esterase	≥ 120 U/L
Peroxidase	25000 U/L
Non-reactive ingredients:	
Buffers, stabilizers and fillers	

Precautions:

Good laboratory safety practices should be followed when handling any laboratory reagent. Refer to a recognized laboratory safety program for additional information. (See GP17-T, Clinical Laboratory Safety; Tentative Guideline (1994), National Committee on Clinical Laboratory Standards, Wayne, PA.)

Intended for in vitro diagnostic use only.

Preparation:

Ready to use liquid reagent, no preparation required.

Storage:

Stable until the expiration date on the label when stored at 2–8 °C. Protect from light and microbial contamination.

Indications of instability:

If the absorbance of the reagent without sample added is over 0.600 at 500 nm, discard the reagent.

If the reagent fails to recover stated values for control sera, discard the reagent.

Discard the reagent after the expiration date on the label.

SPECIMEN

Collection and preparation[2]:

SERUM: Use serum collected by standard venipuncture technique.

PLASMA: Use Heparin or EDTA plasma collected by standard venipuncture technique.

The patient should sit quietly for about 5 minutes before the sample is drawn. A minimum of 10 µL of serum or plasma is required for this assay. The optimum quantity of serum or plasma is 0.5 mL.

Interfering substances:

Bilirubin: No significant interference observed at 20 mg/dL.

Hemolysis: No significant interference observed at 500 mg/dL.

Lipemia: No significant interference observed at 1000 mg/dL.

Young[3,4] has published a comprehensive list of drugs and substances which may interfere with in vitro diagnostic assays, including that of cholesterol.

Storage:

Cholesterol levels in the sample have been reported to be stable for 5-7 days at 4°C or room temperature, 3 months at –20°C, and many years at –70°C.[5,6]

Materials provided:

CHL1100 5 x 60 mL

Materials required but not provided:

Spectrophotometer or colorimeter capable of measuring absorbance at 500 nm.

Matched cuvettes.

Constant temperature incubator set at 30 °C, or 37 °C. Use the same temperature for assay of standard, controls and samples.

Pipettes to measure reagent and samples.

Instructions:

Wavelength: 500 nm

Test: 1 mL reagent + 10 µL sample

Standard: 1 mL reagent + 10 µL standard

Blank: 1 mL reagent

Incubate: 5 minutes at 37 °C

or:10 minutes at 30 °C

Reading: Set instrument to 0 absorbance with the blank. Read absorbance (A) of the test and standard within 3 minutes from the end of the incubation period recommended above.

CALIBRATION

Use a reliable, commercially available standard or calibrator with established values for cholesterol concentrations.

It is recommended that each laboratory establish its own procedures for corrective action if calibration is not acceptable.

QUALITY CONTROL

Serum controls are recommended to monitor the performance of manual and automated assay procedures, providing a continued screening of the instrument, reagents and technique. Commercially available control material with established values for cholesterol concentrations may be used.

RESULTS

C_{st} = Value of standard in mg/dL of cholesterol.

$$\frac{\text{A sample}}{\text{A standard}} \times C_{st} = \text{Cholesterol in sample in mg/dL.}$$

Sample Calculation:

If the absorbance of the sample is 0.235 and that of the standard is 0.470 and the value of the standard is 300 mg/dL:

$$\frac{0.235}{0.470} \times 300 = 150 \text{ mg/dL Cholesterol}$$

LIMITATIONS OF THE PROCEDURE

Samples with cholesterol concentrations exceeding the linearity of this assay (700 mg/dL) should be diluted with an equal volume of physiological

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saline (150 mmol/L sodium chloride in water) and assayed again; multiply results by 2.

Plasma levels may be approximately 3% lower than serum values. It is recommended that if plasma is used, the plasma value should be converted to a serum value according to the following factor:

Serum cholesterol = Plasma cholesterol × 1.03 [2].

PERFORMANCE

Linearity: The assay is linear to 700 mg/dL.

Correlation: Results obtained with this reagent in 107 serum samples, ranging in cholesterol value from 55 to 545 mg/dL, were compared with those obtained using Raichem Cholesterol Reagent. The correlation coefficient was 0.998 and the regression equation was y = 1.006x - 1.422.

Precision: Precision studies conducted in accordance with NCCLS EP5-T2, using this reagent and control serum, at
37 °C, yielded the following:

Within Run		
Mean (mg/dL)	130	239
SD	2.2	1.7
CV (%)	1.7	0.7
N	21	21
Total		
Mean (mg/dL)	130	239
SD	3.0	4.5
CV (%)	2.4	1.9
N	21	21

Sensitivity: 1mg/dL = 0.0016 ΔA

REFERENCE RANGE

The use of risk groups to classify accepted concentrations of total cholesterol in serum have been suggested by recent studies [7,8]. The risk groups have been identified as follows:

Risk Classification	Total Cholesterol
Desirable	< 200 mg/dL
Borderline High	200-239 mg/dL
High	≥ 240 mg/dL

At least two measurements of cholesterol on separate occasions should be made before a medical decision is made. A single point total cholesterol measurement may not represent a patient's usual cholesterol concentration. Cholesterol results that are at the decision points should be followed with a repeat measurement.

It is recommended that each laboratory establish its own reference range.

REFERENCES:

Allain, C.C., Poon, L.S., Clan, C.S.G, Richmond, W. and Fu, P.D., Clin. Chem. 20:470, 1974.

Special Report: Recommendations for Improving Cholesterol Measurement: A Report from the Laboratory Standardization Panel of the National Cholesterol Education Program, NIH Publication No. 90-2964, Feb., 1990.

Young, D.S., Effects of Preanalytical Variables on Clinical Laboratory Tests, Second Edition, 3.120-3.132, AACC Press, Washington, D.C., 1997.

Young, D.S., Effects of Drugs on Clinical Laboratory Tests, 4th Edition, 3.143-3.163, AACC Press, Washington, D.C., 1995.

Tietz, N. W. Clinical Guide to Laboratory Tests, 3rd Edition. Philadelphia, PA, WB Saunders Company 1995:130.

Clinical Chemistry, Principles and Technics, Edited by Henry, Harper and Row, New York, 1974.

Special Report: Current Status of Blood Cholesterol Measurement in Clinical Laboratories in the US.: A Report from the Laboratory Standardization Panel of the National Cholesterol Education Program, Clin. Chem 34:193-201, 1988.

Third Reprot of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III), Executive Summary. NIH Publication No. 01-3670: May 2001.

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