UDI

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CHOLESTEROL (LIQUID) ENZYMATIC

REF 024

FOR IN VITRO DIAGNOSTIC USE

INTENDED USE

For the quantitative determination of total cholesterol in serum / plasma by enzymatic color/endpoint method.

DIAGNOSTIC SIGNIFICANCE

Cholesterol is a fatty substance found in blood, bile and brain tissues. It serves as a precursor to bile acids, steriods and vitamin D. The determination of serum cholesterol is a major aid in the diagnosis and classification of lipemias[1]. Other conditions such as hepatic and thyroid diseases influence cholesterol levels[2].

EXPECTED VALUES[3]

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

RISK CLASSIFICATION	TOTAL CHOLESTEROL (mg/dl)
Desirable	<200
Borderline High	200 - 239
High	≥240

METHOD PRINCIPLE

Enzymatic methods, involving cholesterol esterase and oxidase and Trinders color system have replaced older methodologies. Allain et al developed a total enzymatic technique in which hydrogen peroxide during the oxidation of cholesterol is used in conjunction with peroxidase, 4-aminoantipyrine and phenol to form a quinoneimine dye[4] that absorbs light which is measured at 505 nm.

The enzymatic reaction sequence employed in the assay of cholesterol is as follows:

$$\text{Cholesterol ester} + H_2O \xrightarrow[\text{esterase}]{\text{Cholesterol}} \text{Cholesterol} + \text{Fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow[\text{oxidase}]{\text{Cholesterol}} \text{Cholestene-3-one} + H_2O_2$$

$$2H_2O_2 + \text{phenol} + \text{4-Aminoantipyrine} \xrightarrow{\text{peroxidase}} \text{quinoneimine} + 4H_2O$$

Cholesterol Esters are hydrolyzed to produce cholesterol. Hydrogen peroxide is then produced from the oxidation of cholesterol by cholesterol oxidase. In a coupled reaction catalyzed by peroxidase, quinoneimine red colored dye is formed from 4-aminoantipyrine, phenol and hydrogen peroxide. The absorption of light at 505 ± 5 nm of the solution of this dye is proportional to the concentration of cholesterol in the sample.

REAGENTS

1. CHOLESTEROL (LIQUID) ENZYMATIC REAGENT

4-Aminoantipyrine 0.6 mM, Cholesterol Esterase >150 U/L, Cholesterol Oxidase 200 U/L, Horseradish Peroxidase >1000 U/L, Phenol 25 mmol/L, Sodium Choiate 0.5 mmol/L, Surfactant, Non-reactive stabilizers and fillers.

2. *CHOLESTEROL STANDARD* (200 mg/dL or 5.17 mmol/L) : Cholestrol in alcohol. Keep tightly capped and store at 2 °C to 8 °C

STORAGE AND STABILITY

Store the reagent at 2 °C to 8 °C. Stable up to expiration date indicated on the vial label.

WARNING AND PRECAUTIONS

1. For *in vitro* diagnostic use

CAUTION : Cholesterol (Liquid) Enzymatic Reagent contains Phenol. **Avoid contact.**

2. Specimen should be considered as infectious; handle appropriately.

3. Use distilled or deionized water where indicated.

REAGENT DETERIORATION

The reagent should be discarded if:

1. Turbidity has occurred. Turbidity may be a sign of contamination.

2. Moisture has penetrated the vial and caking has occurred.

3. The reagent fails to meet linearity claims or fails to recover control values in the stated range.

SPECIMEN

SERUM / HEPARINIZED PLASMA.

1. Test specimen should be free from hemolysis.

2. Cholesterol in serum /plasma is reported as stable for seven (7) days at room temperature (15 °C to 25 °C) and six (6) months when frozen (-20 °C) and properly protected against evaporation[5].

MATERIALS PROVIDED

Cholesterol (Liquid) Enzymatic Reagent; Cholestrol Standard (200 mg/dl).

PROCEDURE (AUTOMATED)

Refer appropriate instrument application guide available from us.

PROCEDURE (MANUAL)

Pipette into clean dry test tubes

	BLANK	STANDARD	TEST
Cholesterol (Liquid) Enzymatic Reagent	1.0 ml	1.0 ml	1.0 ml
Pre-warm at 37 °C for 3 minutes and add :			
Standard	--	0.01 ml	--
Sample	--	--	0.01 ml
Mix and incubate at 37 °C for 10 minutes. Read the absorbance of standard and test at 505 ± 5 nm against blank.			

NOTE **:**

If the spectrophotometer being used requires a final volume greater than 1.0 ml for accurate reading, use

0.025 ml (25 µl) of sample to 2.5 ml of reagent. Perform the test as described above.

CALCULATIONS

(A = Absorbance)

$$\frac{A(TEST)}{A(Standard)} \times \text{Conc. of Standard (mg/dl)} = \text{Conc. of TEST (mg/dl)}$$

EXAMPLE: A (TEST) = 0.40, A (STANDARD) = 0.32, Concentration of Standard = 200 mg/dl

$$\text{Conc. of TEST} = \frac{0.40}{0.32} \times 200 = 250 \text{ mg/dl}$$

INTERFERING SUBSTANCES

Anticoagulants, such as fluoride and oxalate will result in false low values[6]. The test is not influenced by hemoglobin values up to 200 mg/dl or by bilirubin levels up to 10 mg/dl. Interference from grossly icteric and heavily hemolysed specimens is correctable by use of a serum/plasma blank.

PROCEDURE LIMITATIONS

This reagent is linear up to 500 mg/dl.

1. Samples with value of above 500 mg/dl should be diluted 1:1 with isotonic saline and re-run. Multiply the final results by two (2).

2. Grossly lipemic serums require a "sample blank". Add 0.01 ml (10 µl) of sample to 1.0 ml saline, mix and read the absorbance against water. Subtract this value from the patient absorbance to obtain the correct reading.

PERFORMANCE CHARACTERISTICS

1. LINEARITY : 500 mg/dl

2. COMPARISON: UDI reagent tested on Manual Systems(y) was compared with CAPS survey results and similar UDI reagent for other systems(x). The systematic difference between the results were within CLIA specified limits. N = 36

Correlation Coefficient	0.989
Regression Equation	y = 1x + 4.34

3. PRECISION

	Mean mg/dL	SD	CV%
Within run	136.2	4.15	3.05
Run to run	130.4	3.16	2.43

4. SPECIFICITY : Cholesterol oxidase is not totally specific for cholesterol. Other analogs of cholesterol (dihydrocholeserol, 7- dehydrocholesterol, 20- hydroxychole-sterol etc.) are also oxidized. These analogs do not normally occur in any appreciable amounts in serum.

QUALITY CONTROL

For accuracy and prsecision check, we recommend the use of normal and abnormal UDI controls based on human serum.

ORDERING INFORMATION :

UDITROL 'N' (Normal Serum Control)
REF # 070N-010 2 x 5 ml
UDITROL 'A' (Abnormal Serum Control)
REF # 070A-010 2 x 5 ml

REFERENCES

1. Beaumount, J.L., Crison, L.A., Cooper, G.R., Feifar, Z., Frederickson, D.S., and Strasser, T.; "Classfication of Hyperlipidemias and Hyperlipoproteinemias". Standard Methods of Clinical Chemistry vol. 9, Academic Press, New York, NY (1972).

2. Holvey, D.N., ed. The Merck Manual of Diagnosis and Therapy.

3. Naito, H.K., et al., Clin. Chem. 20:193 (1988).

4. Allain, C.C., et al., Clin Chem. 20:470 (1974).

5. Tietz, N.W., Fundamentals of Clin. Chem., Pheladelphia, W.B. Saunders (1970).

6. Young, D.S., et al., Clin Chem. 21 No.5 (1975).

PRODUCT AVAILABILITY

CHOLESTEROL (LIQUID) ENZYMATIC

REF # 024-100	**5 x 20 ml**
REF # 024-250	**1 x 250 ml**
REF # 024-500	**2 x 250 ml**

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CE