CHOLESTEROL

(CE-CO-PAP, Enzymatic, End-Point)

Certified ISO 9001-2000

BIO LAB DIAGNOSTICS

CLINICALSIGNIFICANCE

Total Cholesterol : Increased levels are associated with atherosclerosis, nephrosis, diabetes mellitus, myxoedema, obstructive jaundice. Decreased levels are observed in cases of hyperthyroidism, certain anaemias, malabsorption and wasting syndrome.
HDL Cholesterol : Decreased levels are associated with increased risk of developing coronary artery diseases and other atherosclerotic diseases.

PRINCIPLE

In blood, Cholesterol is associated with two kinds of lipoproteins : high density lipoproteins (HDL) and low density lippoteins (LDL and VLDL). Recent studies have shown that the level of Cholesterol bound to HDL is a better indicator of cardiovascular risk than the total cholesterol level, cholesterol esters CE cholesterol + fatty acids

cholesterol + O CO cholestenone3. One+HO

2 H2O2.4AAP.DHBS POD quindye+4H_2O

Composition+

Final concentration in the reactive medium

1. ENZYME

Phosphate buffer	100mmol/L
Cholesterol Esterase	300 U/L
Cholesterol Oxidase	100U/L
Hydroxybenzoicacid	20 mmol/L
4-Aminophenazone	0.5mMol/L
Peroxidase	> 200 U/L
Surface active agents	10g/L
Stabilizers	1g/L

2. BUFFER

Potassium Phospha	100mMol/L

3. STANDARD

Cholesterol (STD) 200 mGs/dL (5.18mMol/L)

REAGENTS SUPPLIED

Cat. No. A 141

1. Enzyme Powder	10mL	10 vials
2. Buffer	10mL	10 Bottles
3. Standard (200 mGs/dL)	4mL	1 Bottle

Cat. No. A140

1. Enzyme Powder	10mL	3 vials
2. Buffer	10mL	3 Bottles
3. Standard (200 mGs/dL)	4mL	1 Bottle

Preparation of Working Reagent
Dissolve on enzyme vial (Reagent No. 1) with 10mL buffer (Reagent No. 2) A uniform solution may take place after5 minutes which is ready to use.
STABILITY
At 2-8℃ for 18 months. The reconstituted reagent is stable for atleast 25 days at 2-8℃ away from light.

SYSTEM PARAMETERS

Reaction	End-Point
Direction of reaction	increasing
Temperature	37℃
Wave Length	510 nM (500-546 nM)
Standard Concentration	200 mg/dL
Absorbance Range	0-2°A
Cuvette Path Length	1 cm
Reaction Time	10mins.
Linearity	700 mg/dL
Max. Limit of Blank Reagent	< 0.100
Final ColourStability	30mins.

Reagent Volume	100µL
Sample Volume	10µL

MANUAL METHOD

1. Pipette into 3 Test Tubes .....
   Cholesterol Reagent No. 1 .....mL
   Distilled water .....mL
   Standard Reagent No. 3 ..... mL
   Sample or HDL Supernatant
   ..... m
   L

Blank	Sample	Standard
1.00	1.00	1.00
0.02	-	-
-	-	-
-	0.02	-

2. Mix well. Incubate at 37℃for 10 minutes or 15 minutes at 30℃ + 5℃.
3. After incubation add 2mL distilled water for 3 mL cuvette size read at 510 nM (500-546 nM) or GREEN filter against Blank. *Final colour is stable upto 30 minutes away from bright light.*

METHOD FORAUTOANALYSERS

Pipette into 3 test tubes .....
Reagent .....mL
Sample ..... mL
Standard.....mL

Blank	Sample	Standard
1.00	1.00	1.00
-	-	0.01
-	0.01	-

Mix well. Incubate for 10 minutes at 37℃. Read the absorbance of sample and standard at 510 nM + 20 against blank reagent and sample standard volume can be altered proportionately.

METHOD FOR HDL CHOLESTEROL

STEP II Pipette into 3 Test Tubes

1. Cholesterol Reagent No. 1 ..... mL
2. HDL Reagent..... mL
3. Supematentfrom STEP I. (given below) .....mL
4. Cholesterol Std (200 mGs/dL) ..... mL

Mix well. Incubate at 37℃ for 10 minutes or 15 minutes at 30℃ + 5℃. After incubation read at 510 nM (500-546nM) or **GREEN** filter against HDL blank. Add 2ml distilled water for 3 mL cuvette size. ***Final colour is stable upto 30 minutes away from bright light.*** **Note** : Standard 200 mGs/dL volume is only 0.02 mL where as sample volume is 0.1 mL.

WARNING

This reagent system is for in vitro Diagnostic use only. This reagent system is containing preservatives and components that have not established for safety if contacted on broken skin or eye or taken orally. In case of such incidents wash off with plenty of water. **METHOD FOR HDL CHOLESTEROL**

STEP I

Serum	**0.2** mL
HDL Reagent	0.05 mL

Mix well, wait for 10 mins. and centrifuge. Separate clear supernatent and estimate cholesterol level of the supermatent asgiveninSTEPII.

RESULTS

Compute Total Cholesterol in mGs/dL =

$$\frac{\text{A O. D. Test}}{\text{SO.D.Std.}} \times 200$$

EXPECTEDVALUES

Total Cholesterol:
150 to 200 mGs/dL (3.88 to 6.47 mMol/L) As with all diagnostic methods, the final diagnosis should not be made on the result of a single test as well as laboratory diagnosis must be confirmed with clinical manifestations **RESULTS**

Compute HDL Cholesterol in mGs/dL = O. D. Test HDL - O.D. HDL Blank

$$\frac{}{\text{O.D. HDL Std. - O.D. HDL Blank}} \times 50$$

This assay is linear up to 700 mg/dL cholesterol. For values higher than this limit dilute the sample with 0.9% normal saline and multiply the results by dilution factor i.e. by 2 for 1:1 dilution. The enzyme system is inactivated by contamination of $AgNO_3$, $HgCl_2$, Teepol or similar substances, and false result may be obtained by the contamination of H_2O_2' Hypochlorite etc.

QUALITY CONTROL

To ensure adequate quality control, each kit should be tested against a standard control sera. It should be realised that the use of quality control material checks both instrument function, temperature control, cleanliness of glass wares and accuracy of pipetting.
It is appropriate to establish each laboratory's accuracy constant and interpret values accordingly. Similarly laboratory findings should be established by clinical manifestations.

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