

Lifechem™ CHOLESTEROL & HDL CHOLESTEROL (CHOD/POD METHOD)

CLINICAL SIGNIFICANCE:

Cholesterol measurements are used in the diagnosis and treatment of lipid / lipoprotein disorders. Lipids play an important role in the body. They serve as hormones, hormone precursors, aid in digestion, provide energy, storage and metabolic fuels, act as functional and structural components in biomembranes and form insulation to allow neuro conduction and prevent heat loss.

Decreased HDL Cholesterol levels are associated with increased risk of developing Coronary Artery Disease and other Atherosclerotic complications.

TEST PRINCIPLE:

The reaction sequence employed in this assay is as follows:



Cholesterol is determined after enzymatic hydrolysis and oxidation. Cholesterol esters are hydrolysed by the enzyme Cholesterol esterase to give free cholesterol and fatty acid molecules. This free cholesterol gets oxidized in the presence of Cholesterol oxidase to liberate Cholest-4 ene-3 one and peroxide. The indicator quinoneimine is formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase. The intensity of this coloured complex is measured at 505 nm (500-540 nm) and is directly proportional to the cholesterol concentration present in the sample.

On addition of the Precipitating reagent to the serum, followed by centrifugation, HDL fraction remains in the supernatant while the other Lipoproteins precipitate out.

NORMAL RANGE:

Total cholesterol : 130 –250 mg/dl (< 6.45 mmol/L)

HDL cholesterol : Male : 30 –70 mg/dl Female : 35-90 mg/dl

It is recommended that laboratories establish their own normal range.

KIT CONTENTS:

| | Code No. KC1 (1x50 ml) | Code No. KC2 (2x50ml) | Code No. KC3 (1x500 ml) |
|---|------------------------------|-----------------------------|-------------------------------|
| Reagent 1 Enzyme Reagent | 1x50 ml | 2 x 50 ml | 1x500 ml |
| Reagent 2 Precipitating Reagent | 5 ml | 10 ml | 50 ml |
| Reagent 3 Cholesterol Standard (200 mg/dl) | 1 ml | 1 ml | 2x1 ml |

SPECIMEN:

Serum/Heparinised or EDTA Plasma

WORKING REAGENT PREPARATION:

The reagent is ready-to-use. All reagents are to be stored at 2-8 °C and are stable till the expiry date mentioned on the labels

PROCEDURE:

Step 1 - Separation of HDL Cholesterol fraction:

Pipette into a centrifuge tube:

| | |
|---------------------------|--------|
| Serum / Plasma | 0.2 ml |
| Precipitating Reagent (2) | 0.3 ml |

Mix well and allow to stand at R.T for 5 minutes. Centrifuge at 3000 rpm for 10 minutes to get a clear supernatant. If the supernatant is not clear (high TGL level) dilute the sample 1:1 with normal Saline and multiply the result with 2.

Step 2 – For Total and HDL Cholesterol :

1.0ml Procedure:

Pipette into 4 test tubes labeled Blank (B), Standard (S), Total Cholesterol (T_c) and HDL Cholesterol (T_H) as shown below:

| | B | S | T _c | T _H |
|---------------------------|--------|--------|----------------|----------------|
| Enzyme Reagent (1) | 1.0 ml | 1.0 ml | 1.0 ml | 1.0 ml |
| Cholesterol Standard(3) | — | 10 μl | — | — |
| Specimen | — | — | 10 μl | — |
| Supernatant (from Step 1) | — | — | — | 100 μl |
| Distilled Water | 0.1 ml | 0.1 ml | 0.1 ml | — |

Mix well and incubate for 5 minutes at 37 °C (or) 10 minutes at R.T. Read the absorbances of Standard (S), Total Cholesterol (T_c) and HDL Cholesterol (T_H) against Blank (B) at 505 nm or with green filter (500 – 540 nm). The final colour is stable for one hour.

CALCULATIONS:

$$\text{a) Total Cholesterol (in mg/dl)} = \frac{\text{Abs. of T}_c}{\text{Abs of S}} \times 200$$

$$\text{b) HDL Cholesterol (in mg/dl)} = \frac{\text{Abs. of T}_H}{\text{Abs of S}} \times 50$$

QUALITY CONTROL:

It is recommended to include Assayed Quality Control Serum (Level 1 & II) with each assay batch to verify the performance of the procedure. Failure to obtain the proper range of values in the assay of control sera may indicate either reagent deterioration, instrument malfunction or procedural errors

SYSTEM PARAMETERS:

| | | | |
|----------------|--------------------|----------------|-----------|
| Mode | : End Point | Std. Conc. | : 200 |
| Wave length | : 505 nm (500-540) | Units | : mg/dl |
| Flow Cell Temp | : 37°C | Blank | : Reagent |
| Sample volume | : 10 μL | Reagent Volume | : 1000 μL |
| Low normal | : 130 | High normal | : 250 |

NOTES:

- The enzyme Reagent on storage at 2-8°C develops a slight pink colouration. However, this does not affect the performance of the test.
- Hemoglobin values upto 200 mg/dl, Bilirubin upto 507 μmol/L and Triglycerides upto 4.64 mmol/L do not interfere with the test.
- Before the assay, bring all the reagents to room temperature.
- Avoid contamination of the reagents during the assay process.
- Programmes for specific autoanalysers are available on request
- As with all the diagnostic procedures, the physician should evaluate data obtained by the use of this kit in light of other clinical information.

LINEARITY:

Linearity of the kit is up to 750 mg/dl.

BIBLIOGRAPHY:

- Richmond, N. (1973), Clin. Chem., 19:1350.
- Abeil, L.L. *et al*, (1952), J. Biol. Chem., 195:357.
- Trinder, P. (1969), Ann. Clin. Biochem., 6:24.



Kamineni Life Sciences Pvt. Ltd

Unit D 4-7, Industrial Estate, Moula-Ali
Hyderabad – 500 040