

CHOLESTEROL & HDL CHOLESTEROL KIT

(CHOD - PAP Method)

For the determination of Cholesterol and HDL Cholesterol in serum.

(For In vitro Diagnostic Use Only)

CLINICAL SIGNIFICANCE

Cholesterol is the main lipid found in blood, bile and brain tissues. It is the main lipid associated with arteriosclerotic vascular diseases. It is required for the formation of steroids and cellular membranes. The liver metabolises the cholesterol and it is transported in the blood stream by lipoproteins.

INCREASES

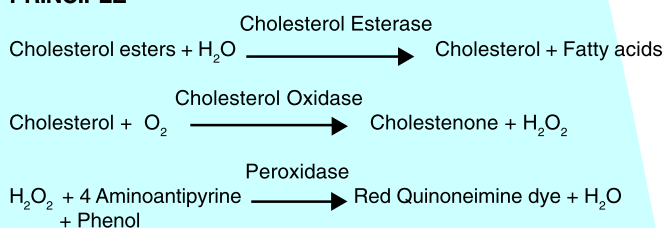
Increased levels are found in hypercholesterolaemia, hyperlipidaemia, hypothyroidism, uncontrolled diabetes, nephrotic syndrome, and cirrhosis.

DECREASES

Decreased levels are found in malabsorption, malnutrition, hyperthyroidism, anemias and liver diseases.

METHODOLOGY – CHOD / PAP method.

PRINCIPLE



Intensity of the colour formed is directly proportional to the amount of cholesterol present in the sample.

REAGENT COMPOSITION

Phosphate Buffer - 100mmol/L Peroxidase - 2.0 KU
Cholesterol esterase - 1.2 KU 4-AminoAntipyrine - 0.4mmol/L
Cholesterol oxidase - 0.9 KU Phenol - 15mmol/L
Activator & Stabilizers

CHOLESTEROL STANDARD - 200mg/dl

HDL CHOLESTEROL STANDARD - 50mg/dl

STORAGE/STABILITY

Contents are stable at 2 - 8°C till the expiry mentioned on the labels.

REAGENT PREPARATION

Reagents are ready to use for the given procedure.

SAMPLE MATERIAL

Serum, EDTA Plasma. Cholesterol is reported to be stable in the sample for 7 days when stored at 2 - 8°C. The sample should preferably of 12 to 14 hours fasting.

ASSAY PARAMETERS

Reaction	End Point	Interval	-
Wavelength	505nm (500-540nm)	Sample Vol.	0.01ml
Zero Settings	Reagent blank	Reagent Vol.	1.00 ml
Incub. Temp	37°C / R.T.	Standard	200 mg/dl
Incub Time	5 min / 10 min	HDL Standard	50 mg/dl
Delay Time	-	React. Slope	Increasing
Read Time	-	Linearity	1000 mg/dl
No. of read.	-	Units	mg/dl

ASSAY PROCEDURE

Wavelength / filter : 505 nm (Hg 546 nm) / Green
Temperature : 37°C / R.T.
Light Path : 1 cm

Pipette into clean dry test tubes labelled as Blank (B), Standard (S) and Test (T).

Step 1 – For HDL Cholesterol :

Pipette into a centrifuge tube :

Serum / Plasma	0.2 ml
Precipitating Reagent (A ₂)	0.3 ml

Mix well 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, (result x 2)

Step 2 – For Total and HDL Cholesterol :

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

Addition Sequence	B (ml)	S (ml)	T _C (ml)	T _H (ml)
Mono Reagent (A ₁)	1.0 ml	1.0 ml	1.0 ml	1.0 ml
Cholesterol Standard (A ₃)	-	10 µl	-	-
Specimen	-	-	10 µl	-
Supernatant (from Step 1)	-	-	-	100 µl

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).

CALCULATIONS

$$1. \text{ Cholesterol in mg/dl} = \frac{\text{Abs.T}_C}{\text{Abs.S}} \times 200$$

$$2. \text{ HDL Cholesterol} = \frac{\text{Abs.T}_H}{\text{Abs.S}} \times 50$$

LINEARITY

This procedure is linear upto 1000 mg/dl. If values exceed this limit, dilute the serum with normal saline (NaCl 0.9%) and repeat the assay. Calculate the value using the proper dilution factor.

The linearity of HDL Cholesterol is 150 mg/dl.

NOTE

Anticoagulants such as fluorides and oxalates result in false low values. The test is not influenced by Hb values upto 20 mg/dl and bilirubin upto 10 mg/dl.

QUALITY CONTROL

To ensure adequate quality control each run should include assayed normal & abnormal controls.

NORMAL VALUES

Total Cholesterol : 130 – 250 mg/dl

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

It is recommended that laboratories establish their own normal range representing its patient population.

REFERENCES

1. Trinder, P., (1969) Ann. Clin. Biochem, 6:24
2. Allain, C.C., etal, (1974) Clin. Chem, 20:470
3. Flegg, H.M., (1972) Ann. Clin. Biochem. 10:79

PRESENTATION

PRODUCT CODE	PACK SIZE	Mono REAGENT A ₁	PRECI-PITATING REAGENT (A ₂)	STANDARD (s)
ACM 0605	1 x 60 ml	1 x 60 ml	1 x 6 ml	1 x 2 ml
ACM 0606	2 x 110 ml	2 x 110 ml	2 x 10 ml	1 x 2 ml

PRODUCT FEATURES AT A GLANCE :

1. Ready to use Reagent.
2. Cholesterol & HDL Cholesterol estimation simultaneously in one test run.
3. Specially Stabilized with proprietary stabilizer.
4. Low blank formulation.
5. Incubation time only 5 minutes at 37°C.
6. Ideal for tropical environment.
7. No interferences from Bilirubin, Triglycerides, and Hemoglobin.
8. Convenient pack sizes –1 x 60 ml & 2 x 110 ml.
9. Shelf life 18 months.
10. Store at 2-8°C.



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IN VITRO DIAGNOSTIC REAGENTS

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