

CHOLESTEROL

(Wybenga & Pileggi) End-Point

BIO LAB
DIAGNOSTICS
ISO 9001:2015
ISO 13485:2016



CLINICAL SIGNIFICANCE

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 hot acidic medium, Cholesterol oxidises ferric ions to a **brown** coloured complex which absorbs at 530 nM and is directly proportional to cholesterol concentration.

REAGENTS COMPOSITION

1. Cholesterol Reagent	250 mL
Acetic Ethyl Acetate	6.5 Mol/L
Sulphuric Acid	3.8 mMol/L
Ferric ion	306 µMol/L
2. Cholesterol Standard	5.0 mL
Cholesterol	200 mGs/dL
Acetic Acid	q.s.
3. HDL Precipitation Reagent	5.2 mL
Phosphotungstate	13.9 nMol/L
MgCl	490 mMol/L

Working Reagent Preparation

All Reagents are ready to use.

STORAGE AND STABILITY

Reagents are stable for 18 months at room temperature away from light.

SAMPLE

Sample can be serum of plasma which has no sign of haemolysis. Common anticoagulants have no interference on this assay. Cholesterol is affected by food intake. Hence, keep the patient fasting for at least 8 hrs. prior to sample collection. (All samples should be handled as potential infective agents as no laboratory methods make conclusive findings for its safety. Therefore, adequate protective laboratory measures should be taken while handling such materials).

WARNING

This reagent system is for in vitro use only. This reagent system contains 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 or consult a physician.

MANUAL PROCEDURE : FOR TOTAL CHOLESTEROL

- Pipette into 3 Test Tubes
Cholesterol Reagent No.1mL
Distilled watermL
Standard Reagent No. 2mL
SamplemL

Blank mL	Sample mL	Standard mL
5.00	5.00	5.00
0.05	-	-
-	-	0.05
-	0.05	-

- Mix well for 20 secs. Keep in a boiling water bath immediately for exactly 90 seconds (1½ minutes). Cool immediately for 5 minutes under running tap-water. Read at 520-540 nM or GREEN filter against Blank. *The final colour is stable for 30 minutes.*

STEP I : FOR HDL-CHOLESTEROL : (Precipitation)

Serum **0.2 mL**
HDL Reagent No. 3 **0.2 mL**

Mix well. Keep for 10 minutes and centrifuge. Separate Clear supernatant and estimate Cholesterol level of the supernatant as per **STEP II**.

STEP II : FOR HDL CHOLESTEROL

- Pipette into 3 Test Tubes

Cholesterol Reagent No. 1mL
HDL Reagent No.3mL
Supernatant from STEP I.mL
Cholesterol Std (200 mGs/dL)mL

Blank HDL mL	Std HDL mL	Test HDL mL
5.00	5.00	5.00
0.20	0.20	-
-	-	0.20
-	0.02*	-

**Standard (200 mGs/dL) volume is only 0.02 mL where as sample volume is 0.2 mL.*

- Mix well for 20 seconds. Immediately keep in a boiling water bath for exactly 90 seconds (1½ minutes). Cool immediately for 5 minutes under running tap water. Read at 520-540 nM **GREEN** filter against HDL blank. *(Final colour is stable for 30 minutes).*

EXPECTED VALUES

Total Cholesterol : 150 to 250 mGs/dL
(3.88 to 6.47 mMol/L)

HDL Cholesterol :

Men : 30 to 60 mGs/dL
(0.78 to 1.55 mMol/L)

Women : 40 to 70 mGs/dL
(1.03 to 1.81 mMol/dL)

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.

LIMITATIONS

Cholesterol Reagent is highly corrosive and viscous. **Elevated results** are obtained when pipetting is not accurate due to high viscosity of the reagent. **Low readings** are obtained due to contamination of reagent by water from the glassware.

This assay is linear upto 500 mGs/dL. For higher values sample must be diluted with 0.9% sodium chloride and the result multiplied by dilution factor e.g. by 2 for 1:1 dilution.

RESULTS

$$\text{Total Cholesterol} = \frac{\text{O. D. Test}}{\text{O. D. Std.}} \times 200$$

$$\text{HDL Cholesterol} = \frac{\text{O. D. Test}}{\text{O. D. Std.}} \times 40$$

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 and reagent function together. Factors which might affect the performance of this test include proper instrument function, temperature control, cleanliness of glasswares 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.

BIBLIOGRAPHY

- BURSTEIN, M.SCHOLNICK, H.R., MORFIN, R.J. of Lipids Res. (1970) 11,583.
- LOPES VIRELLAM., STONE P., ELLIS S., ORWELL J. A. Clin. Chem. 1977, 23, 882.
- GROVE T.H. Clin. Chem. (1997), 25, 560.
- N. TIETZ, Ed. Fundamentals of Clin. Chem. WYBENGA et al.

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