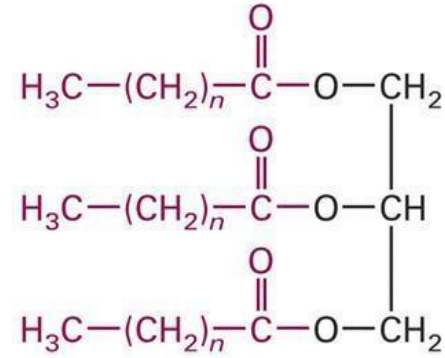
Triglyceride determination

Introduction:

- Triglycerides are esters of fatty acids and are hydrolyzed to glycerol and free fatty acids (by lipase)

- Triglyceride determinations when performed in conjunction with other lipid assays are useful in the diagnosis of **primary and secondary hyperlipoproteinemia**.

- They are also of interest in following the course of diabetes mellitus, nephrosis, biliary obstruction, and various metabolic abnormalities due to endocrine disturbances.



Triacylglycerol

- **Hyperlipoproteinemia:** abnormally elevated of fat in blood (disorder in lipid metabolism).

- Standard methods for the measurement of triglyceride concentrations involved either **enzymatic** or **alkaline hydrolysis** to liberate glycerol.

- Principle:

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

Triglycerides + H_2O —(Lipase)→ Glycerol + Fatty Acids

Glycerol + ATP —(Glycerol Kinase)→ Glycerol-3-Phosphate + ADP

Glycerol-3-Phosphate + O_2 —(G-1-P oxidase)→ DAP + H_2O_2

H_2O_2 + 4AAP + 4 chlorophenol —(Peroxidase)→ Quinoneimine Dye + $2H_2O$

- The present procedure involves hydrolysis of triglycerides by lipase.
- The glycerol concentration is then determined by enzymatic assay coupled with Trinder reaction that terminates in the formation of a quinoneimine dye.
- **The amount of the dye formed**, determined by its absorption at 505 nm, is directly proportional to the concentration of triglycerides in the samples.

- Specimen collection and storage:

1. Fresh, non-hemolyzed serum from fasting patients is recommended.

2. Triglycerides in serum appears stable for three days when stored at 2-8 ◦C.

3. Prolonged storage of the samples at room temperature is not recommended since other glycerol containing compounds may hydrolyze, releasing free glycerol with an apparent increase in total triglycerides content.

- Method :

- By Triglyceride reagent kit.

-Follow the table:

	Blank	Standard	Test
Reconstituted Reagent	1 ml	1 ml	1 ml
Pre-worm at 37◦C for 2 min and add:			
Standard **Sample**	--- ---	0.01 ml (10 µl) ---	--- 0.01 ml (10 µl)
Mix and incubate at 37ºC for 10 min ⬇ **Read the absorbance of standard and sample at 505 nm against blank**			

-Calculation:

$$\text{Conc. of TG} = \frac{\text{Ab Test}}{\text{Ab Std.}} \text{ X conc. of Std. (200 mg/dl)}$$

- **Normal range:** 10 -190 mg/dl

HDL-Cholesterol determination

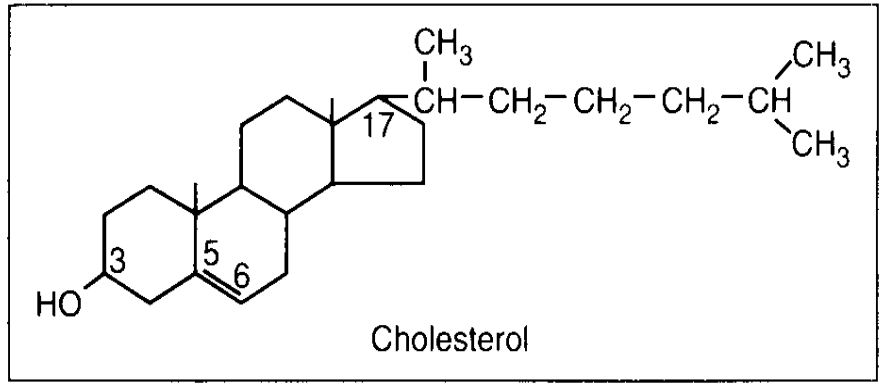
- Introduction:

- Cholesterol is a fatty substance found in blood, bile and brain tissue.

- It serves as a precursor to **bile acids, steroids and vitamin D**.

- In the plasma, cholesterol is transported by three lipoproteins: high density lipoprotein (HDL-Cholesterol), low density lipoprotein (LDL-Cholesterol), and very low density lipoprotein (VLDL- Cholesterol).

- The concentration of **total cholesterol** in serum has been associated with metabolic, infectious and coronary heart diseases.



Cholesterol

- The concentration of HDL-cholesterol in serum has important in diagnosis of the how the level of **risk to get coronary heart diseases**.

- (More HDL-Chol. That indicate low risk to get coronary heart disease.)

- Castelli and co-workers have indicated that an **inverse relationship** exists between serum HDL-Cholesterol and the risk of coronary heart disease.

- The measurement of **HDL Cholesterol and triglyceride** provides valuable information for the prediction of coronary heart disease and for lipoprotein phenotyping.

- Specimen collection:

1. Specimen should be serum and free from hemolysis.

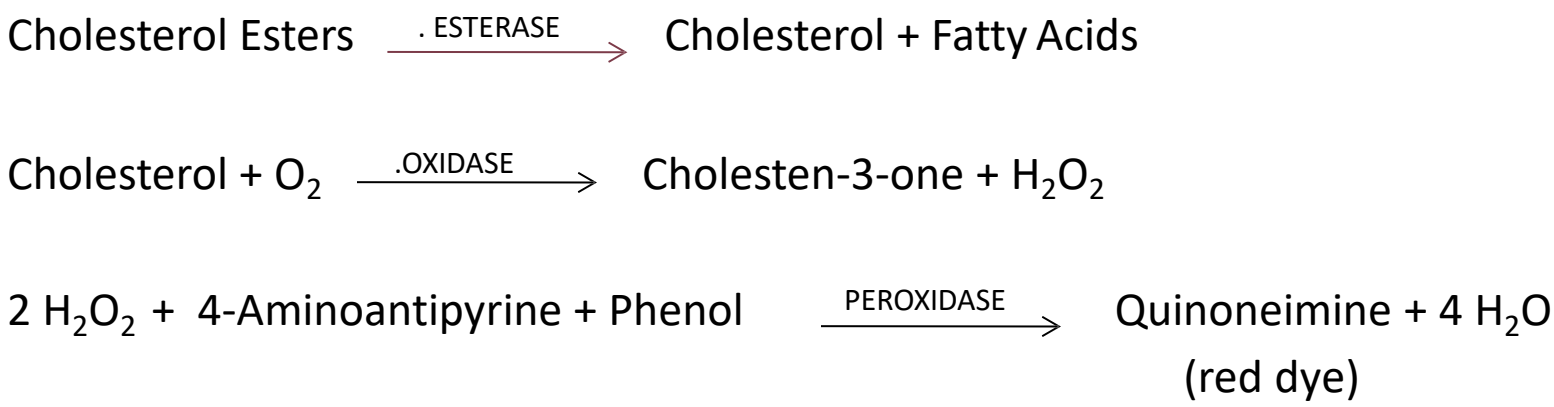
2. Patient should be fasting for 12-14 hours.

- Principle:

- HDL cholesterodetermination

- Enzymatic methods, involving cholesterol esterase and oxidase and Trinders color system.

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



- 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 $\pm$ 5 nm of the solution of this dye is proportional to the concentration of cholesterol in the sample.

Method :

- HDL Cholesterol:

- Follow the Table:

	Blank	Calibrator	Assay
Reagent R1	300 µl	300 µl	300 µl
Calibrator		3 µl	3 µl
Mix vigorously, let stand for 5 min at 37◦C . Read absorbance A1 at 600 nm against blank.			
Add Reagent R2	100 µl	100 µl	100 µl
Mix vigorously, let stand for 5 min at 37◦C . Read absorbance A2 at 600 nm against blank.			

- Calculation :

* **Determine the HDL Cholesterol conc.**

$$\Delta \text{ Abs.} = (A2 - 0.75\ A1)$$

$$\text{Conc. Of HDL} = \frac{\Delta \text{ Ab Assay}}{\Delta \text{ Ab Calibrator}} \text{ X conc. of calibrator (50 mg/dl) = mg/dl}$$

- Normal value of :

- HDL-Cholesterol :

- Low level (risk factor) < 40 mg/dl
- High HDL (protector factor) ≥ 60 mg/dl