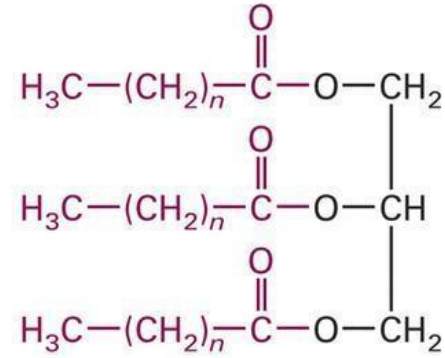
Triglyceride determination

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 500 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:			
Distilled water	0.01 ml (10 µl)		
Standard	---	0.01 ml (10 µl)	
Sample	---	---	0.01 ml (10 µl)
Mix and incubate at 37ºC for 5 min ⬇ **Read the absorbance of standard and sample at 500 nm against blank**			

-Calculation:

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

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

HDL-Cholesterol determination

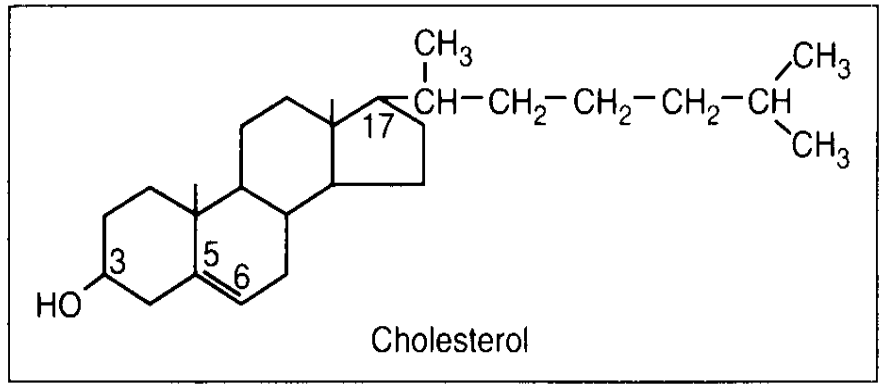
- Introduction:

- 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

- It is direct method without specimen pre treatment.

LDL, VLDL, Chylomicrons $\xrightarrow[\text{DSBmT + Peroxidase}]{\text{Accelerator + CO}}$ Non-Reactive LDL, VLDL, Chylomicrons

HDL-C + Selective Detergent $\xrightarrow[\text{Cholesterol oxidase}]{\text{Cholesterol esterase}}$ Cholestenone + H_2O_2

H_2O_2 + 4-AAP + DSBmT $\xrightarrow{\text{Peroxidase}}$ Color development

- A color reaction which is proportional to HDL-Cholesterol concentration. **The absorbance is measured at 600 nm.**

LDL= Low density lipoprotein

VLDL= Very low density lipoprotein

CO= Cholesterol Oxidase

4-AAP= 4- Aminoantipyrine

POD= Peroxidase

CE= Cholesterol Esterase

AAO= Ascorbate Oxaldase

DSBmT= N,N-bis (4-sulphobutyl)-m-toluidine-disodium

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.**

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

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

- Normal value of:

- HDL-Cholesterol :

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