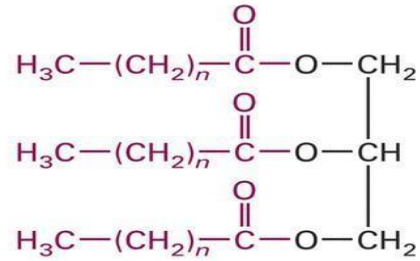
BCH 447

Triglyceride Determination in Serum

Introduction:

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



Triacylglycerol

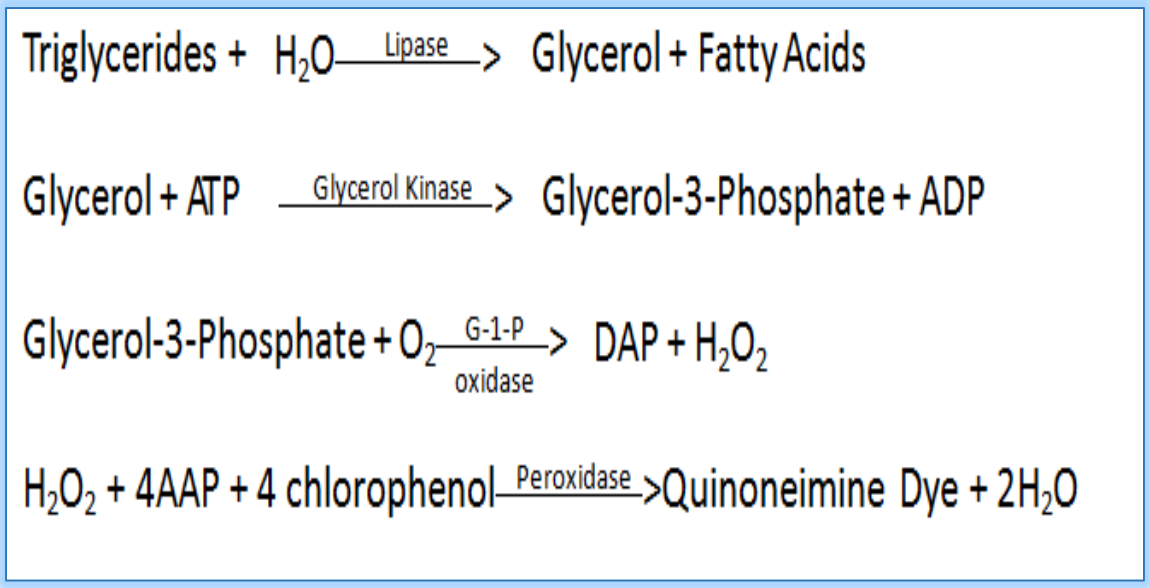
- Triglyceride determinations when performed in conjunction with other lipid assays are useful in the diagnosis of **primary and secondary hyperlipidemia (**abnormally elevated of fat in blood **)**

Principle:

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

•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 is** determined by its absorption at 505 nm, it is directly proportional to the concentration of triglycerides in the samples.



Trinder reaction: It is the reaction between hydrogen peroxide and the phenol and aminoantipyrine (AAP) to form quinoneimine (red-violet dye), catalyzed by the presence of a peroxidase

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-warm at 37◦C for 2 min and add:			
Standard	---	0.01 ml (10 µl)	---
Sample	---	---	0.01 ml (10 µl)
Mix and incubate at 37°C 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

TG → **Hyperthyroidism**

Malnutrition

Low-fat diet

Malabsorption

TG → **Hyperlipidemia**

Hypothyroidism

Obesity

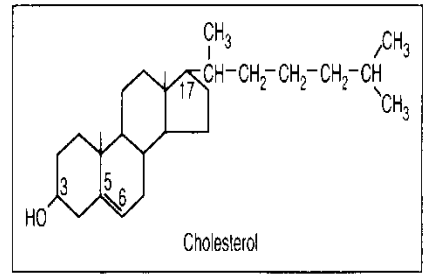
Kidney Disease

Diabetes

HDL-Cholesterol determination

Introduction:

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



Cholesterol

- 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.

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

• There is an **inverse relationship** between serum HDL-Cholesterol and the risk of coronary heart disease

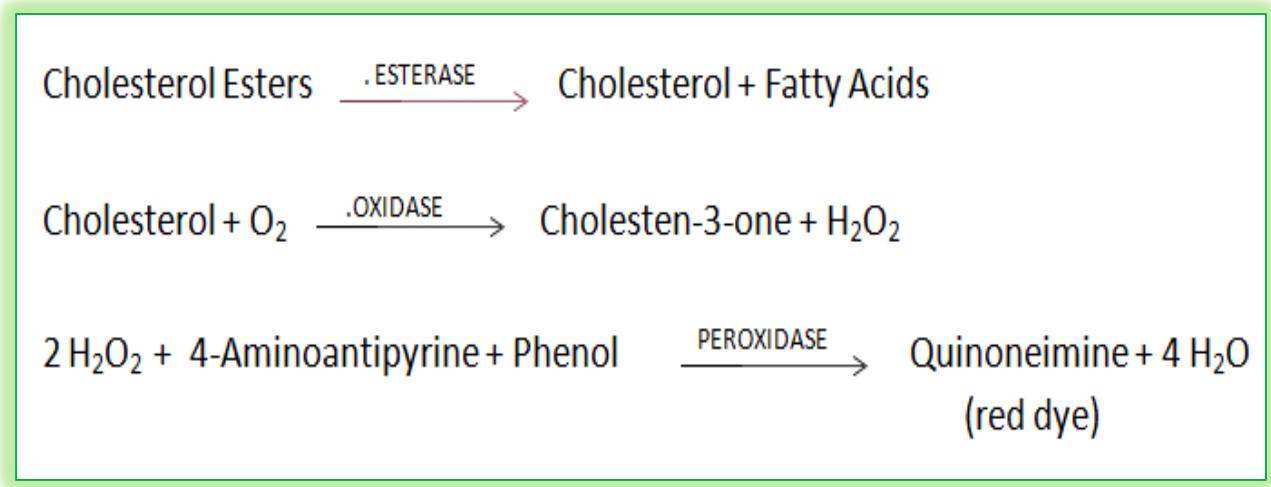
- **More HDL-Chol** → indicate **low risk** of coronary heart disease.

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

Principle:

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

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



•**The amount of the dye formed is** determined by its absorption at 505± 5 nm, it is directly proportional to the concentration of cholesterol in the samples.

Method :

- By HDL-Cholesterol reagent kit.

-Follow the table:

	Blank	Calibrator	Assay
Reagent R1	300 µl	300 µl	300 µl
Calibrator /Sample		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 :

Δ Abs. = (A2 - 0.75 A1)

Conc. of HDL = $\dfrac{\Delta \text{ Ab Assay}}{\Delta \text{ Ab Calibrator}}$ 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