جامعة الملك سعود
King Saud University
1957

Triglyceride Determination in Serum

Introduction

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

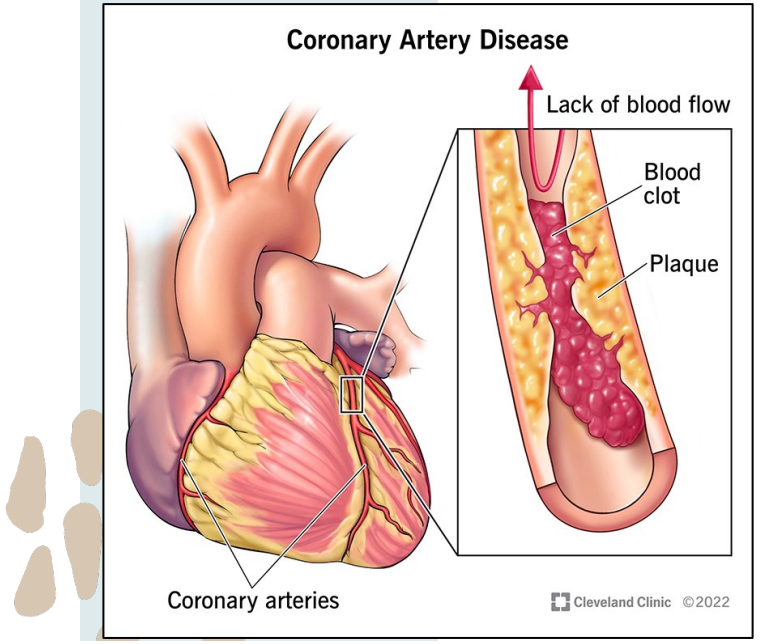


Figure 1. Illustration of the heart with partially-blocked coronary vessels

Triglycerides

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

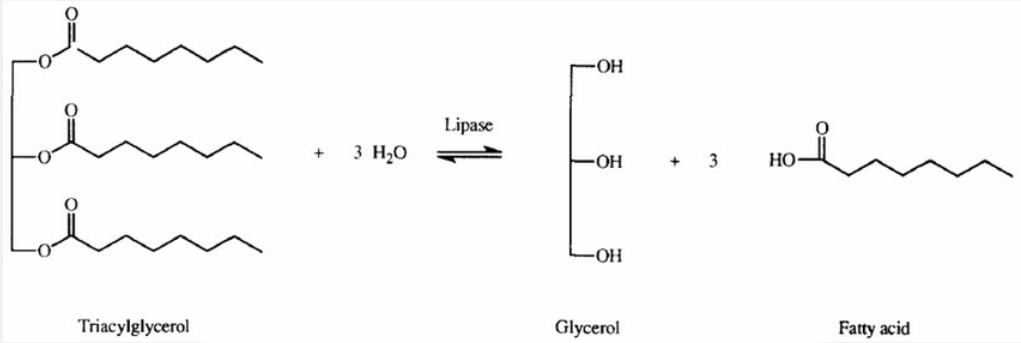


Figure 2. Triacylglycerol hydrolysis reaction

- 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*)

Figure 3. Lipemic blood sample

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.

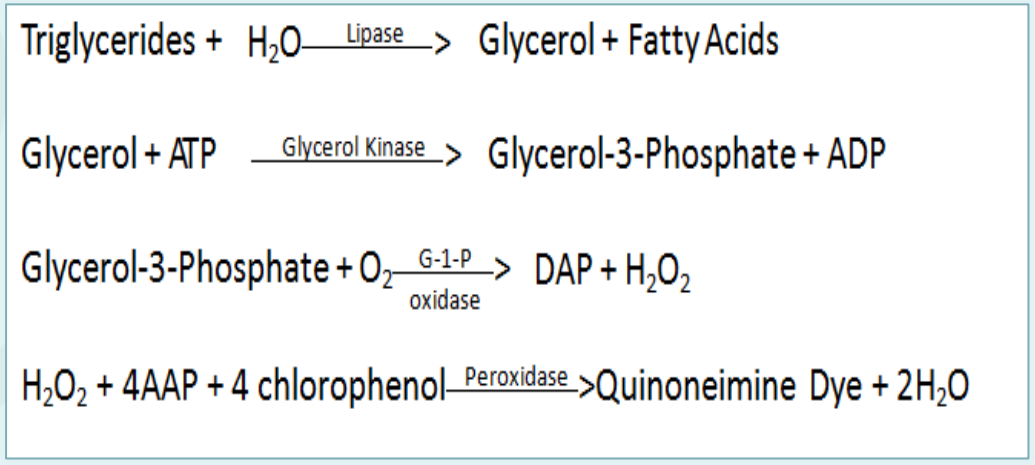


Figure 4. Trinder reaction

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:

- Fresh, non-hemolyzed serum from fasting patients is recommended.
- Triglycerides in serum appears stable for three days when stored at 2-8 ◦C.
- 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.

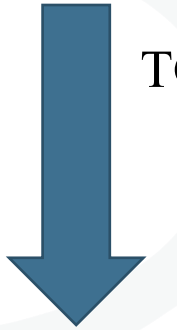
-Follow the table:

	Blank	Standard	Test
Reconstituted Reagent	1 ml	1 ml	1 ml
Pre-warm at 37◦C for 2 min and add:			
Standard (200 mg/dl)	---	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 of TG: 10 -190 mg/dl

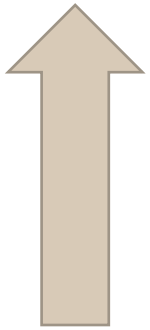


TG → **Hyperthyroidism**

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.
- 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**), good cholesterol , transports cholesterol from tissues to liver.
  - Low density lipoprotein (**LDL-Cholesterol**), bad cholesterol , transports cholesterol from liver to tissues.
  - Very low density lipoprotein (**VLDL- Cholesterol**).

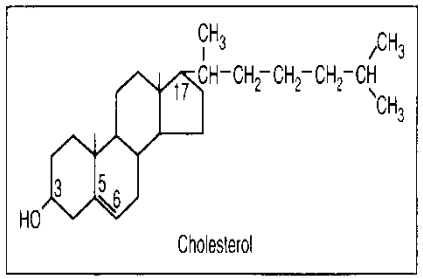


Figure 5. Cholesterol structure

- 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.

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:

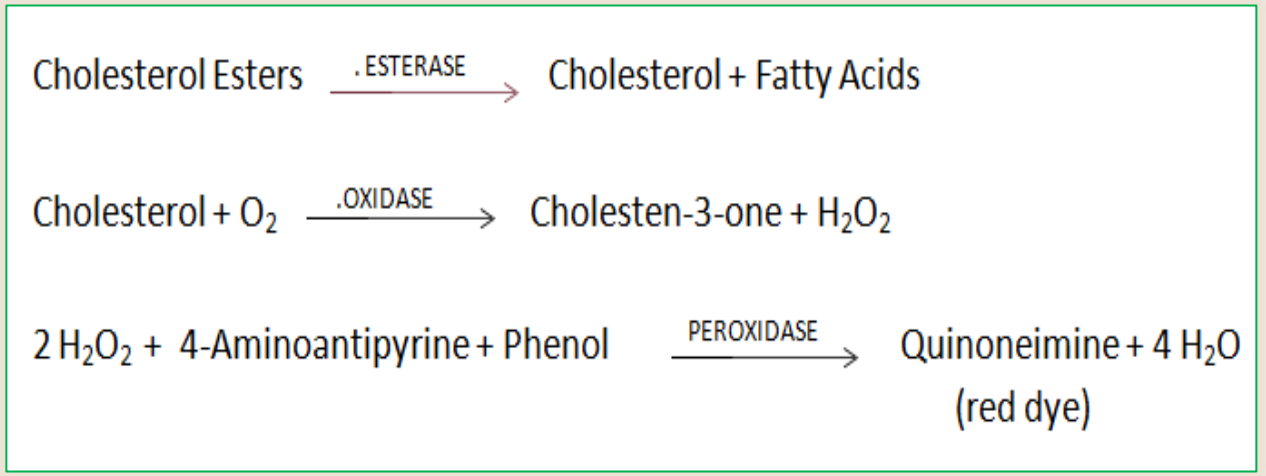


Figure 6. Trinder reaction

- **The amount of the dye formed is** determined by its absorption at 600 nm, it is directly proportional to the concentration of cholesterol in the samples.

Method:

- By HDL-Cholesterol reagent kit.

-Follow the table as in the labsheet

Calculation:

$$\text{Conc. of HDL} = \frac{\text{A2-A1 Test}}{\text{A2-A1 calibrator}} \text{ X conc. of calibrator (90 mg/dl)} = \text{-------- mg/dl}$$

- Normal value of HDL-Cholesterol :

- Low level (risk factor) < 40 mg/dl

- High HDL (protector factor) ≥ 60 mg/dl

Homework:

- **What are the different tests performed in a lipid panel?**
- **Why are fasting samples preferred for lipid profile?**