Cihan University - Sulaimaniya

Clinical Biochemistry

Lab. 10

Measurement of Triglyceride (TG)

Prepared by :

Darya Shorsh Hamad

Mcs. in Clinical Biochemistry

E-mail: darya.shorsh@sulicihan.edu.krd

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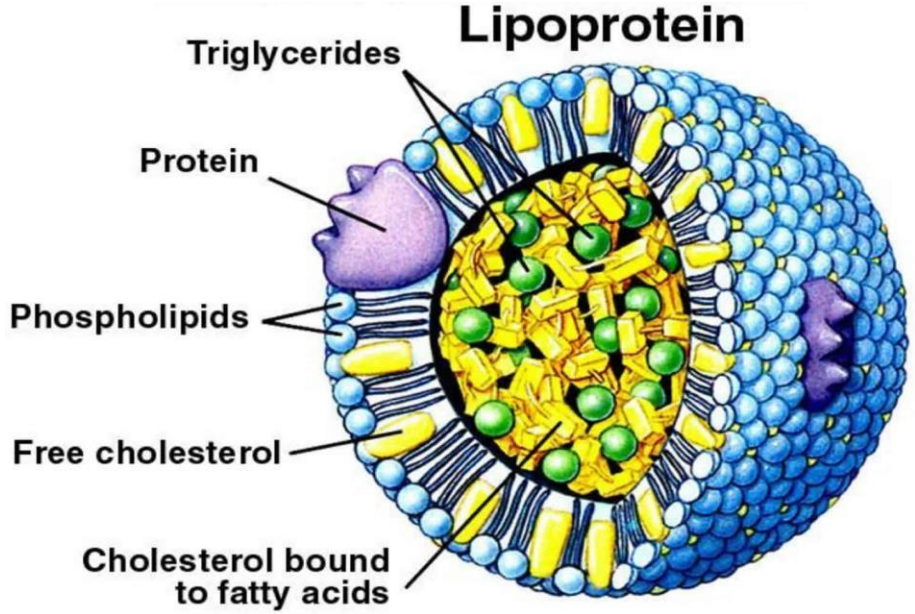
Introduction

Blood lipids

Concept: All the lipids contained in plasma, including fat, phospholipids, cholesterol, cholesterol ester and fatty acid.

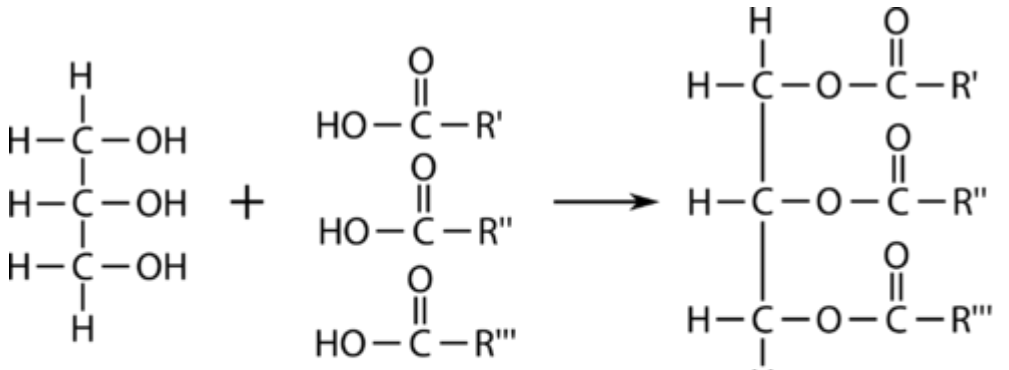
- **blood lipids**
  - **TG**
  - **cholesterol**
    - **free**
    - **ester**
  - **phospholipids**
    - **lecithin**
    - **sphingolipids**
    - **cephalin**
  - **FFA**



Blood lipid exist and transport in the form of **lipoprotein**.

Triglycerides (TG)

- Glycerol backbone with FA attached by ester bonds
- Sources of Triglycerides:
  - Exogenous source: Dietary
  - Endogenous : Liver and tissue storage



glycerol + 3 fatty acids → triglyceride (triester of glycerol)

Clinical Significance of TG Measurement

The measurement of TG concentration in the blood important to diagnosis and following up of Hyperlipidemia

- **Cause of Hyperlipidemia :**
  - Primary: Genetic Origin
  - Secondary:
    - Diabetes Mellitus
    - Hypothyroidism

Friedewald equation

VLDL-c = Triglycerides/5

LDL-c = Total cholesterol - (HDL-c + VLDL-c)

- **Effects of Hyperlipidemia** :
  - Hypertriglyceridemia increases the risk for pancreatitis.
  - Hypertriglyceridemia is associated with the following clinical findings: xanthoma, Lipemia retinalis (LR), hepatomegaly, splenomegaly and decresased HDL-cholesterol.

Procedure

PRINCIPLE (4) (5)

Fossati and Prencipe method associated with Trinder reaction.
Reaction scheme is as follows:

$$\text{Triglycerides} \xrightarrow{\text{Lipase}} \text{Glycerol} + \text{free fatty acids}$$

$$\text{Glycerol} + \text{ATP} \underset{}{\overset{\text{GK}}{\rightleftharpoons}} \text{Glycerol 3 Phosphate} + \text{ADP}$$

$$\text{Glycerol 3 Phosphate} + O_2 \overset{\text{GPO}}{\rightleftharpoons} \text{DihydroxyacetonePhosphate} + H_2O_2$$

$$H_2O_2 + \text{4-Chlorophenol} + \text{PAP} \xrightarrow{\text{POD}} \text{Quinoneimine (pink)} + H_2O$$

The absorbance of the coloured complex (quinoneimine), proportional to the amount of triglycerides in the specimen, is measured at 500 nm.

Reagents

REAGENTS

Vial R1 BUFFER

PIPES	100	mmol/L
Magnesium chloride	9.8	mmol/L
Chloro-4-phenol	3.5	mmol/L
Preservative		

Vial R2 ENZYMES

Lipase	≥ 1000	IU/L
Peroxydase (POD)	≥ 1700	IU/L
Glycerol 3 phosphate oxydase (GPO)	≥ 3000	IU/L
Glycerol Kinase (GK)	≥ 660	IU/L
4 - Amino – antipyrine (PAP)	0.5	mmol/L
Adenosine triphosphate Na (ATP)	1.3	mmol/L

Vial R3 STANDARD

Glycerol	2.28	mmol/L

Equivalent to trioleine or **triglycerides 200 mg/dL** (2.28 mmol/L)

Procedure

REAGENTS PREPARATION

Vial R2: Use a non-sharp instrument to remove aluminium cap.

Add promptly the contents of vial R2 (Enzymes), into vial R1 (Buffer).

Mix gently and wait for complete dissolution before using reagent (approximately 2 minutes).

Procedure

MANUAL PROCEDURE

Let stand reagent and specimens at room temperature.

Pipette into well identified test tubes:	Blank	Standard	Assay
Reagent	1 mL	1 mL	1 mL
Demineralised water	10 µL		
Standard		10 µL	
Specimen			10 µL

Mix. Let stand for 5 minutes at 37°C or 10 minutes at room temperature.
Record absorbance at 500 nm (480-520) against reagent blank.
Reaction is stable for 1 hour.

Calculation

CALCULATION

Calculate the result as follows:

$$\text{Result} = \frac{\text{Abs (Assay)}}{\text{Abs (Standard)}} \times \text{Standard concentration}$$

Ranges for lipid profile Test

LIPID PROFILE

	DESIRABLE	BORDERLINE	HIGH RISK
Cholesterol	<200 mg/dl	200-239 mg/dl	240 mg/dl
Triglycerides	<150 mg/dl	150-199 mg/dl	200-499 mg/dl
HDL cholesterol	60 mg/dl	35-45 mg/dl	<35 mg/dl
LDL cholesterol	60-130 mg/dl	130-159 mg/dl	160-189 mg/dl
Cholesterol/ HDL ratio	4.0	5.0	6.0