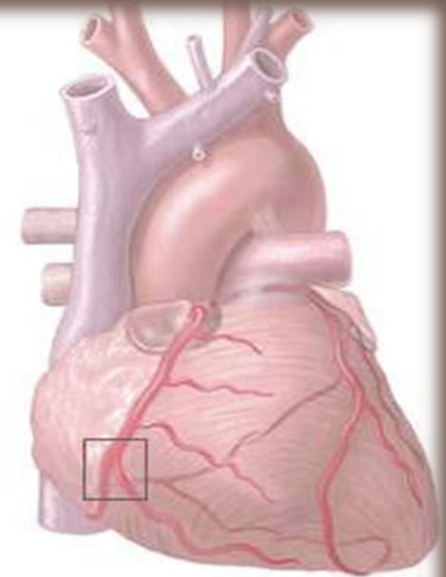


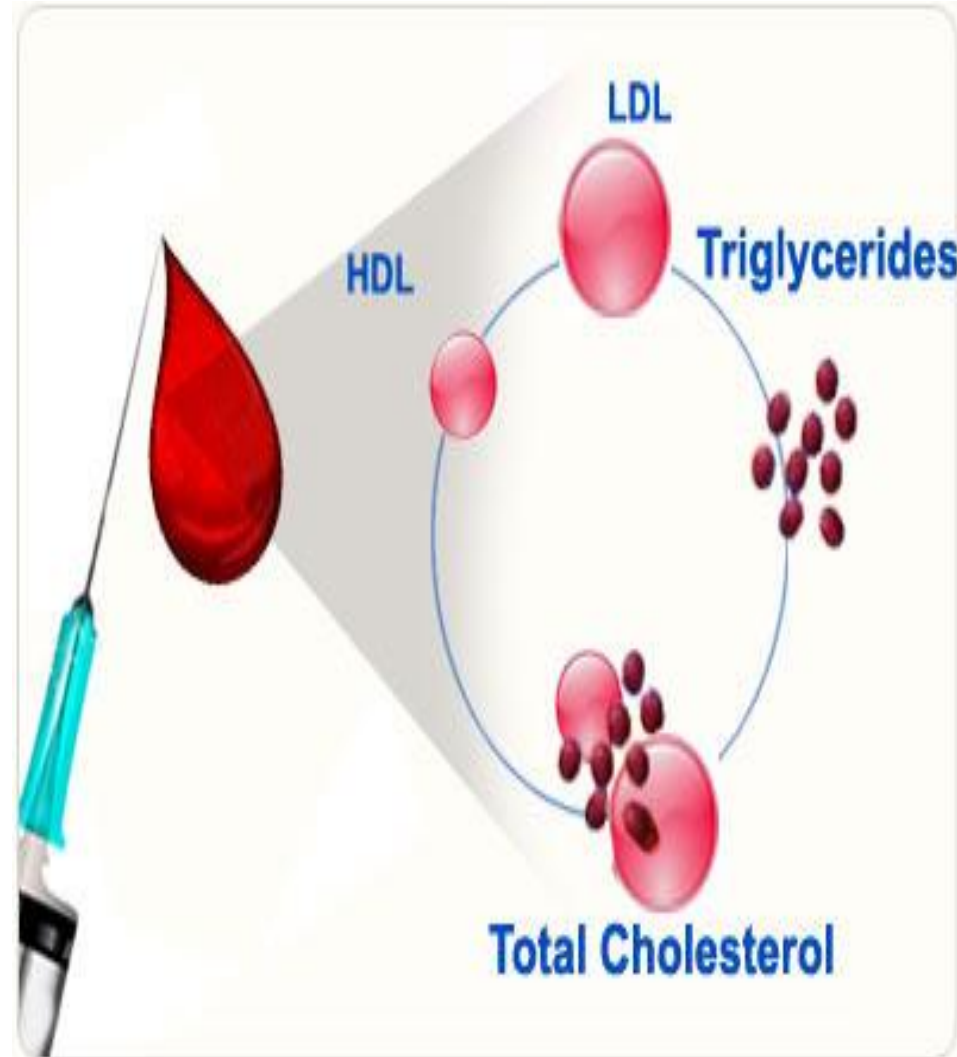
LIPID PROFILE TESTS



ADAM

❑ **Lipid profile or lipid panel**, is the collective term given to the estimation of, typically, total cholesterol, high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), and triglycerides.

❑ An extended lipid profile may include very low-density lipoprotein (VLDL).



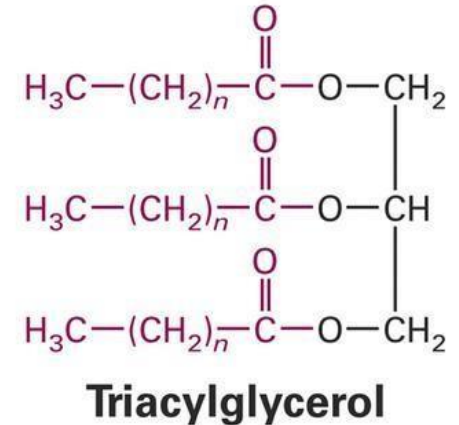
Results of Lipid profile

are used to identify hyperlipidemia (various disturbances of cholesterol and triglyceride levels), many forms of which are recognized risk factors for cardiovascular disease and certain forms of pancreatitis, and other diseases.



Triglycerides TG:

- Triglycerides are esters of fatty acids and are hydrolyzed to glycerol and free fatty acids (by lipase)
 - Triglyceride is body storage form of fat and energy
 - Most TG found in adipose tissue
- Give energy in case of absence of carbohydrates for muscles to work.
- Extra triglycerides are found in the blood after after meal TG "gut" >>>> blood>>>> adipose



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

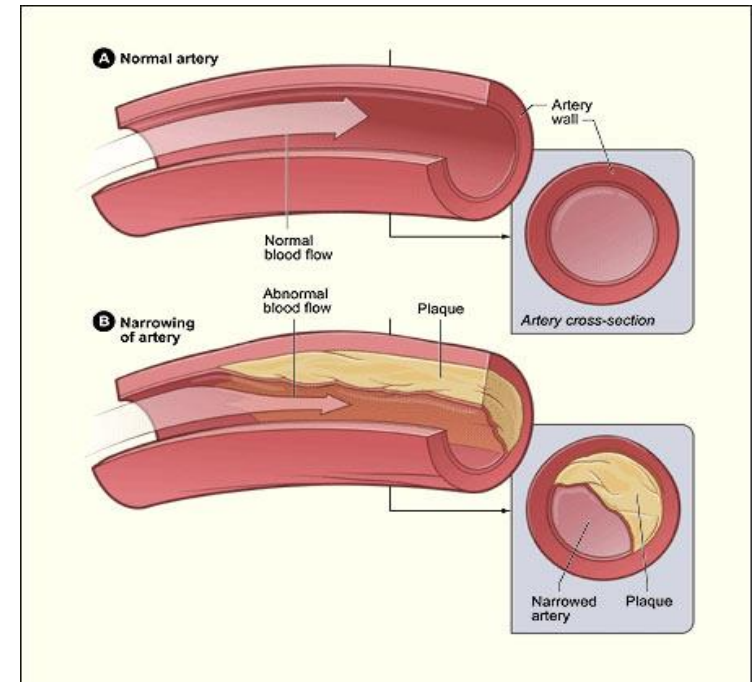
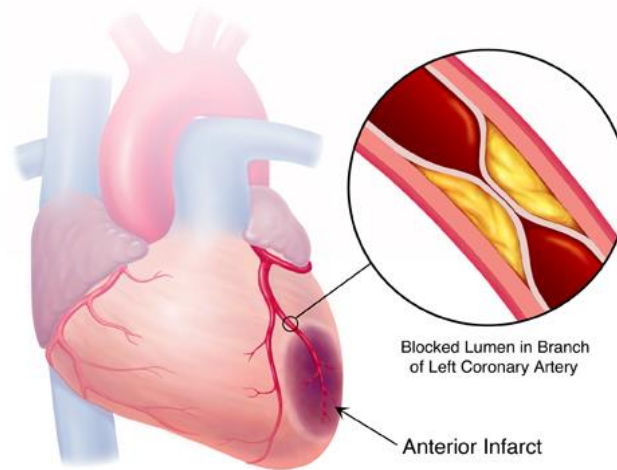
☐ Elevated in obese or diabetic patients. Level increases from eating simple sugars or drinking alcohol. Associated with heart and blood vessel disease.

HYPERLIPIDEMIA

- Is the condition of abnormally elevated levels of any or all lipids and/or lipoproteins in the blood. It consider a heterogeneous group of disorders.



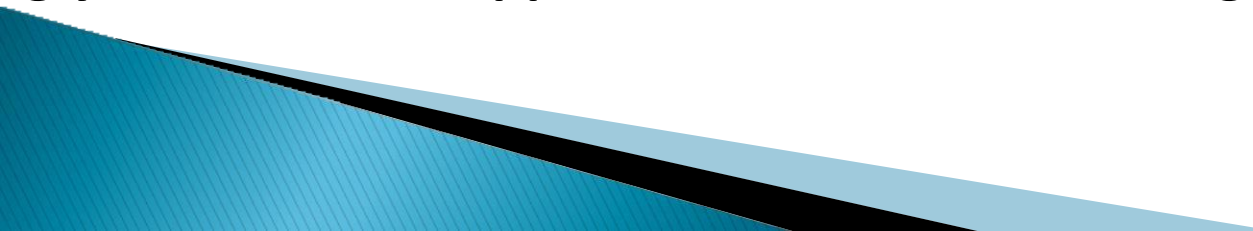
blood (left for 4h)
LDL >40 mmol/L
markedly abnormal



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

-TG test needs 12 hrs fasting because its level is effected by meal (fatty meal, high carbohydrates meal)

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.**
- 

- **Primary hyperlipidemias** are probably genetically based, but the genetic defects are known for only a minority of patients
- **Secondary hyperlipidemia** may result from diseases such as diabetes, thyroid disease, renal disorders, liver disorders, and Cushing's syndrome, as well as obesity, alcohol consumption, estrogen administration, and other drug-associated changes in lipid metabolism
- **Hyperlipidemia** is a major, modifiable risk factor for atherosclerosis and cardiovascular disease, including coronary heart disease; this is true both of disorders involving hypercholesterolemia and hypertriglyceridemia

Hypertriglyceridemia

FACTORS THAT CONTRIBUTE TO ELEVATED SERUM TRIGLYCERIDES

- Excess weight or obesity
- Physical inactivity
- Stress
- Excessively high carbohydrate diets (<60% of the caloric intake)
- Type II diabetes
- Chronic renal failure
- Drugs (such as corticosteroids, estrogens, retinoids, high doses of beta adrenergic blocking agents)
- Certain genetic metabolic disorders (including familial combined hyperlipidemia, and familial hypertriglyceridemia).

- Principle:

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



- The present procedure involves hydrolysis of triglycerides by lipase.
- The glycerol concentration is then determined by enzymatic assay coupled with Trinder reaction that terminates 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.

- 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	---	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:

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

- Normal range:

Less than 150-160 mg/dl

NORMAL RANGE

Men: 40-160 mg/dl (0.45-1.81 mmol/L)

Women: 35-135 mg/dl (0.40-1.53 mmol/L)

Hypertriglyceridemia:

Suspicious: > 150 mg/dl (>1.7 mmol/L)

Elevated: > 200 mg/dl (>2.3 mmol/L)

Cholesterol:

- Is steroid directly linked to heart and blood vessels disease
- It is synthesized in the liver and also comes from the foods you eat (animal products).
- **Has important function in body:**
 - Cholesterol is a fatty substance found in blood, bile and brain tissue.
 - important part in membrane of cells, organs and tissues in the body
 - It serves as a precursor to bile acids, steroids and vitamin D.

Therefore, cholesterol deficiency is not good.

- Source: 70% synthesized in body (liver),
30% from food (animal source as meat, eggs and dairy products)

Cholesterol levels:

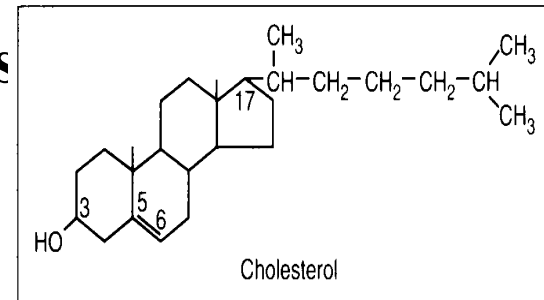
- High level associated with heart disease
- Good level:** below 200 mg/dl (low risk of heart disease)
- Border line:** 240mg/dl (if higher at high risk)

- Notes:

- Measuring blood cholesterol level not need fasting?**

Cholesterol level is not affected by single meal but affected by long term pattern of eating (change from high fat diet to low fat diet for several weeks)

- Cholesterol level is elevated during pregnancy (till 6 weeks after delivery)**
- Some drugs are known to increase cholesterol levels as anabolic steroids, beta blockers, epinephrine, oral contraceptives and vitamin D.**



- The cholesterol **level** in serum is dependent on age, sex, geographical /cultural region and nutrition.
- At birth, the cholesterol level is usually below **100 mg/dl**(2.6 mmol/l).
- Levels increase slowly throughout childhood and generally throughout life.
- This increase is greater in men than in women during the reproductive years.

A- Raised levels may occur in:

- An inherited form of high cholesterol (hypercholesterolemia or hyperlipidemia).
- Heart disease
- Diabetes mellitus.
- Nephrotic syndrome.
- Myxoedema (associated with hypothyroidism).
- Late pregnancy.
- Liver disease.
- Cholesterol is sometimes deposited in the skin as yellow nodules or xanthemas and in arteries (atheroma).

B- Low levels occur in:

- **Severe Infection**
- **Severe Anemia.**
- **Massive Liver Cell Damage.**

Risk Factors of High Cholesterol

- **A Family History**
- **Age**
- **Gender**
- **Diet**
- **Cigarette Smoking**
- **Obesity**
- **Medical Conditions: Diabetes, High BP**
- **Stress**



Lipoproteins



- - lipid are insoluble substances,
- Lipid transported in plasma as lipoproteins**
- in the plasma, cholesterol is transported by **three lipoproteins**: high density lipoprotein (HDL-Cholesterol), low density lipoprotein (LDL-Cholesterol), and very low density
- Triglyceride is transported as chylomicron and VLDL
-

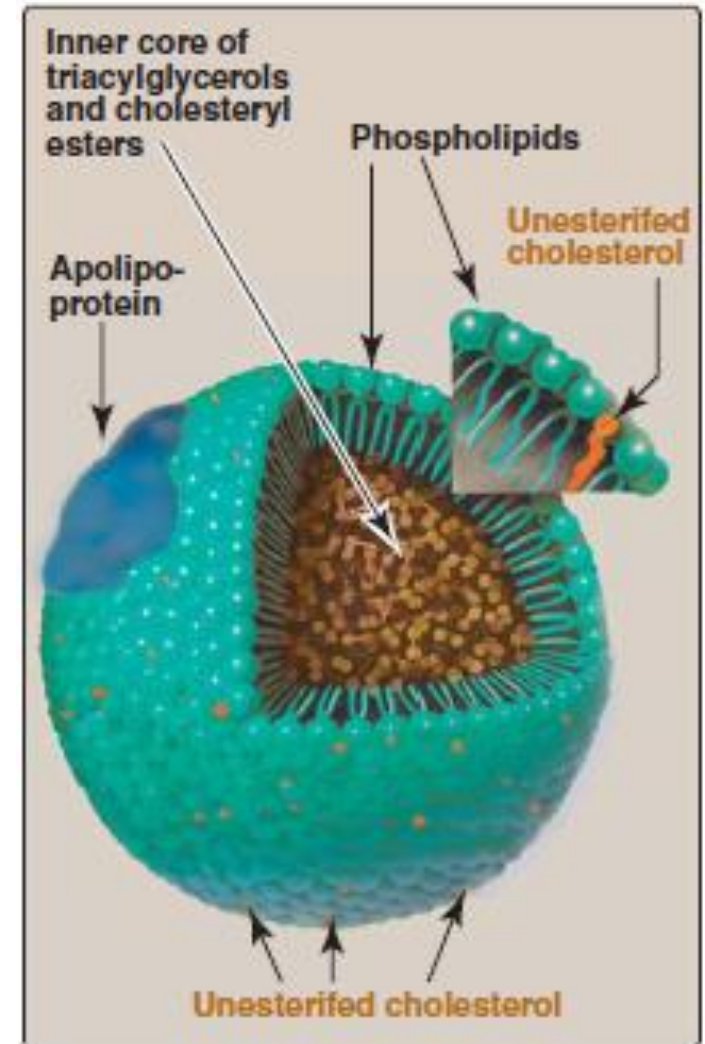
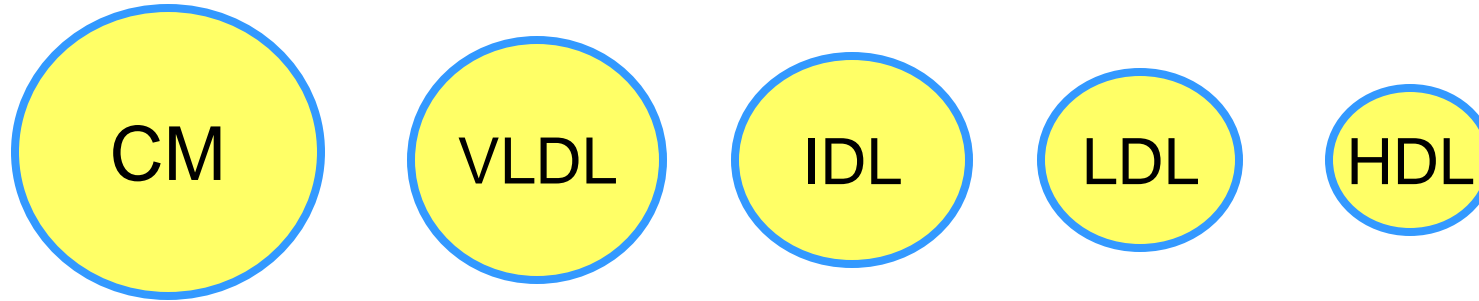


Figure 18.14
Structure of a typical lipoprotein particle.

Lipoprotein Nomenclature and Composition



Major
Protein

apoB

apoB

apoB

apoB

apoA-I

Major
Lipid

TG

TG

CE

CE

CE

CM= chylomicron

VLDL= very low density lipoprotein

IDL= intermediate density lipoprotein

LDL= low density lipoprotein

HDL= high density lipoprotein

Apo = apolipoprotein

TG=triglyceride

CE= cholesteryl ester

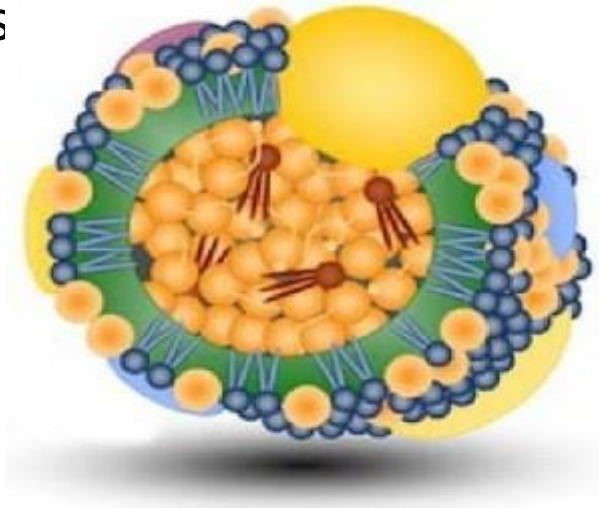
Table I-15-1. Classes of Lipoproteins and Important Apoproteins

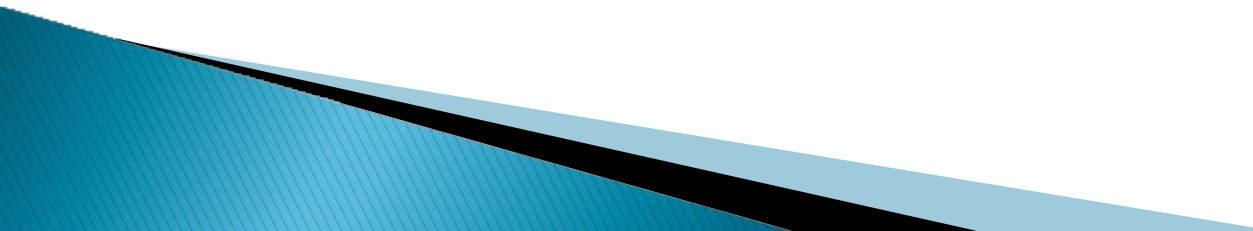
Lipoprotein	Functions	Apoproteins	Functions
Chylomicrons	Transport dietary triglyceride and cholesterol from intestine to tissues	apoB-48 apoC-II apoE	Secreted by intestine Activates lipoprotein lipase Uptake of remnants by the liver
VLDL	Transports triglyceride from liver to tissues	apoB-100 apoC-II apoE	Secreted by liver Activates lipoprotein lipase Uptake of remnants (IDL) by liver
IDL (VLDL remnants)	Picks up cholesterol from HDL to become LDL Picked up by liver	apoE apoB-100	Uptake by liver
LDL	Delivers cholesterol into cells	apoB-100	Uptake by liver and other tissues via LDL receptor (apoB-100 receptor)
HDL	Picks up cholesterol accumulating in blood vessels Delivers cholesterol to liver and steroidogenic tissues via scavenger receptor (SR-B1) Shuttles apoC-II and apoE in blood	apoA-1	Activates lecithin cholesterol acyltransferase (LCAT) to produce cholesterol esters



HDL (high density lipoprotein) :

- **HDL**: good cholesterol, carry cholesterol from organs and blood to liver to get rid of it
- It removes excess cholesterol from tissues (it cleans blood).
- High levels linked to a reduced risk of heart and blood vessel disease. The higher your HDL level, the better.



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

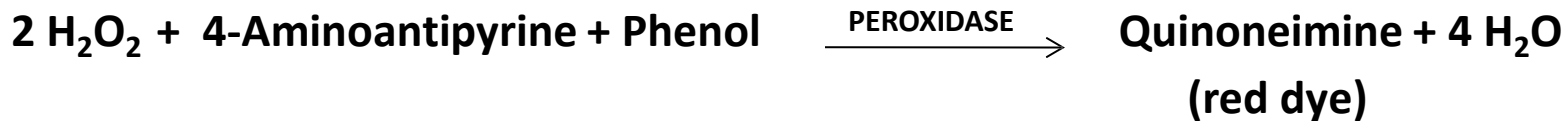
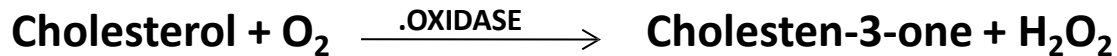
- Specimen collection:

1. Specimen should be serum and free from hemolysis.
2. Patient should be fasting for 12-14 hours.

- Principle:

- HDL cholesterol determination

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

- Preparing HDL-Cholesterol sample:

- When serum is reacted with the polyethylene glycol reagent, all the low and very low-density lipoproteins (LDL and VLDL) are precipitated.
- **The HDL fraction remains in the supernatant.**
- The supernatant is then used as a sample for cholesterol assay.

method

	Blank	Standard	Test
Cholesterol liquid enzymatic reagent	1 ml	1 ml	1 ml
Pre-worm at 37°C for 2 min and add:			
Distilled water	100 µl	---	---
Standard (50 mg/dl)	---	100 µl	---
Supernatant (serum)	---	---	100 µl

Mix and incubate at 37°C for 10 min.

Read Ab. at 505nm against blank.

Method :

- HDL Cholesterol:

- Follow the Table:

	Blank	Standard	Test
Cholesterol liquid enzymatic reagent	1 ml	1 ml	1 ml
Pre-worm at 37°C for 2 min and add:			
Distilled water	100 µl	---	---
Standard (50 mg/dl)	---	100 µl	---
Supernatant (serum)	---	---	100 µl
Mix and incubate at 37°C for 10 min. ↓ Read Ab. at 505nm against blank.			

- Calculation :

* Determine the HDL Cholesterol conc.

$$\text{Conc.} = \frac{\text{Ab Test}}{\text{Ab Std.}} \times \text{conc. of Std (50mg/dl)}$$

- Normal value of :

- HDL-Cholesterol :

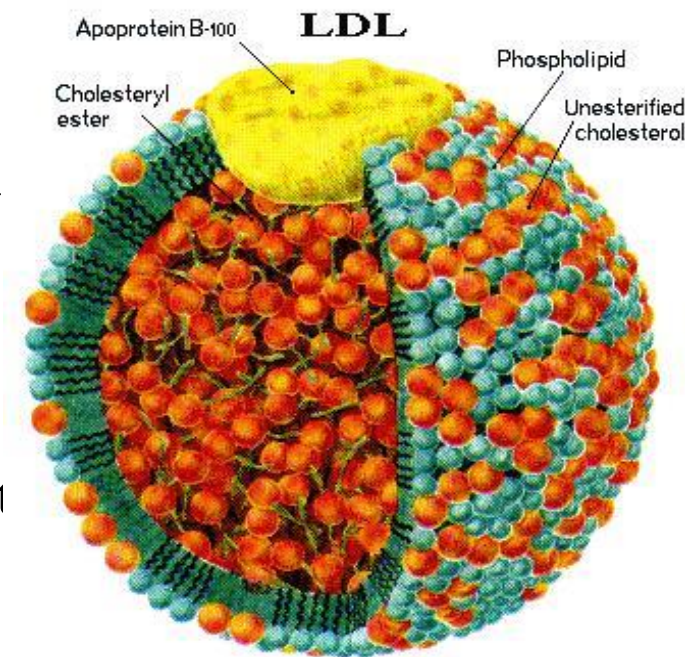
- female > 45 mg / dl

- Male > 35 mg/dl

1- LDL (low density lipoprotein):



- LDL: bad cholesterol " carry cholesterol from liver to blood then to organs**
- It has less protein content and contains more cholesterol**
- LDL cholesterol is easy to stick to the walls of blood vessels.**
- High LDL in blood associated with atherosclerosis, heart disease and myocardial infraction**
- Reducing LDL levels is a major treatment target for cholesterol-lowering medications.**
- Because high LDL in blood will deposited in blood artery and trigger clot formation**



Preparation:

Blood should be collected after a 12-hour fast (no food or drink, except water). For the most accurate results, wait at least 2 months after a heart attack, surgery, infection, injury or pregnancy to check LDL levels.

Normal values:

- Less than 70 mg/dL for those with heart or blood vessel disease and for other patients at very high risk of heart disease (those with metabolic syndrome)**
- Less than 100 mg/dL for high risk patients (e.g., some patients who have multiple heart disease risk factors) if you have heart disease or diabetes.**
- Less than 130 mg/dL for individuals who are at low risk for coronary artery disease . if you have 2 or more risk factors.**
- LDL less than 160 mg/dL if you have 0 or 1 risk factor.**

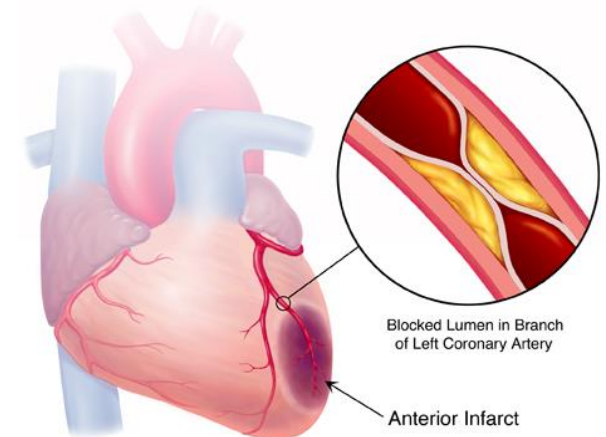
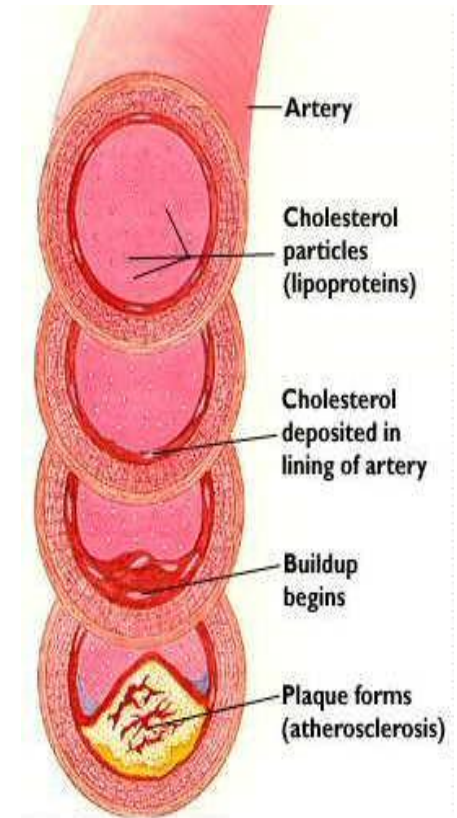
- Less than 130 mg/dL for individuals who are at low risk for coronary artery disease . if you have 2 or more risk factors.**
- LDL less than 160 mg/dL if you have 0 or 1 risk factor.**

Measuring LDL-C level:

- LDL level calculated either : directly or by equation**
- LDL= Total cholesterol - (HDL+TG/5)**

Risk of high LDL and heart disease

- High blood LDL will deposit cholesterol in the inner walls of the arteries that feed the heart and brain.
- It can form plaque (thick, hard deposit) that can narrow the arteries and make them less flexible.
- This condition is known as atherosclerosis.
- If a clot forms and blocks a narrowed artery, heart attack or stroke can result.



- LDL cholesterol values are most often calculated as the amount of cholesterol not contained in HDL and VLDL.
- VLDL is estimated by $TG \div 5$ because the cholesterol concentration in VLDL particles is usually $\frac{1}{5}$ of the total lipid in the particle.
- Thus, LDL cholesterol = $TC - [HDL \text{ cholesterol} + (TGs \div 5)]$ (Friedewald formula). This calculation is valid only when TGs are < 400 mg/dL and patients are fasting, because eating increases TGs.
- The calculated LDL cholesterol value incorporates measures of all non-HDL, nonchylomicron cholesterol, including that in IDL and lipoprotein (a) [Lp(a)].

Case I

A 66-year-old male patient, known case of Diabetes since last 12 years and Coronary Artery Disease for the last 2 years presented in Hospital with the complaints of Chest pain and breathlessness for the last 6 hours and chest pain . Patient had an episode of vomiting. He was conscious and well oriented. Overall health state was weak. .

At physical examination ,he had a heart rate of 90 bpm and blood pressure of 110/70 mmHg. Lung examination showed no alterations total cholesterol is 320 mg/dl

1- Which cardiac biomarkers are elevated?

2-What the biochemical test(s) will you be request from biochemistry lab to confirm your diagnosis?

2- Are there any further biochemical test(s) may help in your diagnosis?