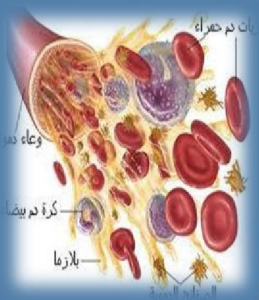
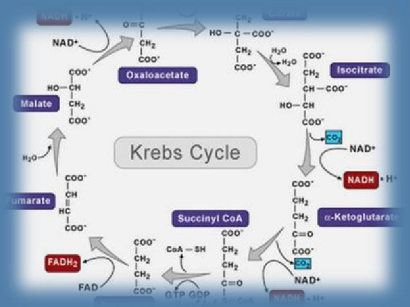
Al-Rasheed University College
Medical Analysis Department
Clinical Chemistry Lab.
Third Stage

كليـــــة الرشيد الجامعـــــــة
قســـــم التحليلات المرضيـــة
مختبر الكيميـــاء السريريـــة
المرحلـــــة الثالثـــــة





Lecture (9)

Lipid Profile Tests:
- Estimation of Blood Triglyceride
- Estimation of HDL-Cholesterol

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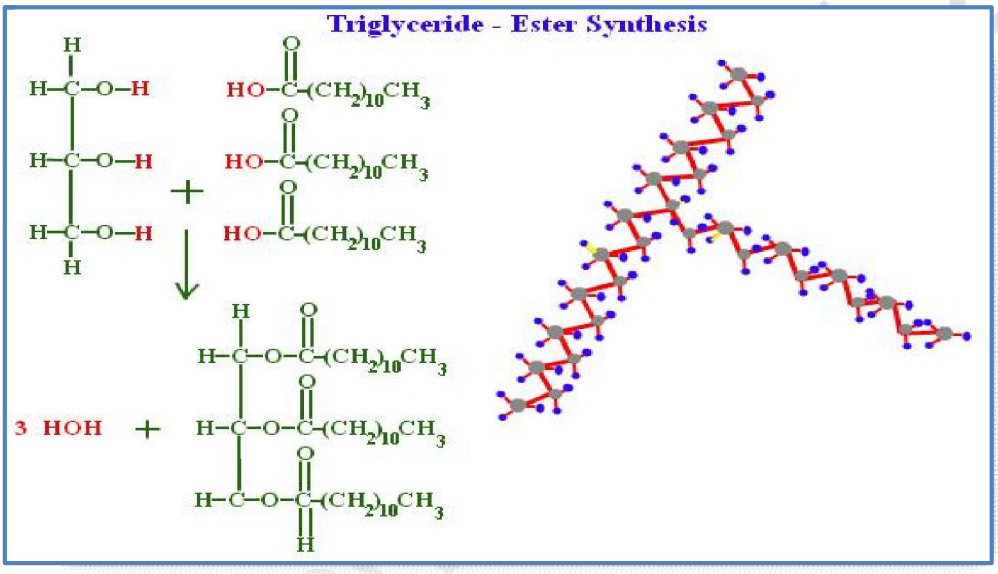
Blood Triglyceride Test

Clinical Significance:

A triglyceride are a type of fat which is an ester derived from glycerol and three fatty acids. As a blood lipid, it helps enable the bidirectional transference of adipose fat and blood glucose from the liver. There are many triglycerides: depending on the oil source, some are highly unsaturated, some less so.



The triglyceride level is a lab test to measure the amount of triglycerides in your blood.

Your body makes some triglycerides. Triglycerides also come from the food you eat. Extra calories are turned into triglycerides and stored in fat cells for later use. If you eat more calories than your body needs, your triglyceride level may be high. A test for high blood cholesterol levels is a related measurement.

The most important use of this test is to help estimate your LDL cholesterol. This test is also done to help assess your risk for heart disease. A high triglyceride level may lead to atherosclerosis. This condition increases your risk of heart attack and stroke. A high triglyceride level may also cause inflammation of your pancreas.

Persons with a high triglyceride level often have other conditions, such as diabetes and obesity, that also increase the risk of getting heart disease.

Test Requirements:

You should not eat for 8 to 12 hours before the test. Alcohol and some medicines can interfere with blood test results.

Normal Results:

Normal: Less than 150 mg/dL

Borderline High: 150 - 199 mg/dL

High: 200 - 499 mg/dL

Very High: 500 mg/dL or above

Abnormal Results Mean:

High triglyceride levels may be due to:

- Cirrhosis or liver damage
- Diet low in protein and high in carbohydrates
- Hypothyroidism (underactive thyroid)
- Nephrotic syndrome (a kidney disorder)
- Poorly controlled diabetes

Low triglyceride levels may be due to:

- Low fat diet
- Hyperthyroidism (overactive thyroid)
- Malabsorption syndrome (conditions in which the small intestine does not absorb fats well)
- Malnutrition.

The principle method:

The principle method of triglyceride test is associated with the following reaction:

$$\text{Triglyceride} \xrightarrow{\text{Lipase}} \text{Glycerol} + \text{free fatty acid}$$

$$\text{Glycerol} + \text{ATP} \xrightleftharpoons{\text{GK}} \text{Glycerol 3-phosphate} + \text{ADP}$$

$$\text{Glycerol 3-phosphate} + O_2 \xrightleftharpoons{\text{GPO}} \text{Dihydroxyacetone phosphate} + H_2O_2$$

$$H_2O_2 + \text{4-Clorophenol} \xrightarrow{\text{POD}} \text{Quinoneimine (Pink)} + H_2O$$

Procedure:

➲ Assay conditions:

- Wavelength …… 500 nm.
- Cuvette ……... 1 cm light path.
- Reference value: men (40 – 160 mg/dl), women (35-135 mg/dl).
- Standard concentration = 200 mg / dl.

	Blank	Standard	Sample
Working Reagent	**1 ml**	**1 ml**	**1 ml**
Standard		**10 µL**	
Sample			**10 µL**

- Mix and incubate for 5 min at (37 ºC) or 10 min at (15-25 ºC).
- Read the absorbance (A) of the samples and standard, against the Blank. The color is stable for at least 30 minutes.

HDL-Cholesterol Test

Clinical Significance:

Cholesterol is a waxy substance (un aqueous substance) that can't dissolve in the blood. that's found in all of your cells and has several useful functions, including helping to build your body's cells. It's carried through your bloodstream attached to proteins. These proteins are called lipoproteins, which got their name because they're made of fat (lipid) and proteins.

The two types of lipoproteins that carry cholesterol to and from cells are ***low-density lipoprotein***, or LDL, and ***high-density lipoprotein***, or HDL. LDL cholesterol and HDL cholesterol, along with one fifth of your triglyceride level, make up your total cholesterol count, which can be determined through a blood test.

- **Low-density lipoproteins (LDL):** These lipoproteins carry cholesterol throughout your body, delivering it to different organs and tissues. But if your body has more cholesterol than it needs, the excess keeps circulating in your blood. Over time, circulating LDL cholesterol can enter your blood vessel walls and start to build up under the vessel lining. Deposits of LDL cholesterol particles within the vessel walls are called plaques, and they begin to narrow your blood vessels. Eventually, plaques can narrow the vessels to the point of blocking blood flow, causing coronary artery disease. This is why LDL cholesterol is often referred to as "bad" cholesterol.
- **High-density lipoproteins (HDL):** These lipoproteins are often referred to as HDL, or "good," cholesterol. They act as cholesterol scavengers, picking up excess cholesterol in your blood and taking it back to your liver where it's broken down. The higher your HDL level, the less "bad" cholesterol you'll have in your blood.

Just lowering your LDL cholesterol might not be enough for people at high risk of heart disease. Increasing HDL cholesterol also can reduce your risk of heart disease.

❹ The test Ordered:

HDL-Cholesterol may be ordered as a follow-up test to a high result on a cholesterol screening test. HDL-Cholesterol is usually not ordered by itself but with other tests, including cholesterol, LDL cholesterol , and triglycerides as part of a lipid profile during a health checkup. It is recommended that all adults be tested at least once every five years.

HDL-Cholesterol, as part of the lipid profile, may be ordered more frequently for those who have one or more risk factors for heart disease. Major risk factors include:

- Cigarette smoking
- Age (men 45 years or older or women 55 years or older)
- Hypertension (blood pressure of 140/90 or higher or taking high blood pressure medications)
- Family history of premature heart disease (heart disease in an immediate family member—male relative under age 55 or female relative under age 65)
- Pre-existing heart disease or already having had a heart attack
- Diabetes mellitus

❹ Abnormal Results Mean:

For adults:

- If HDL-Cholesterol is less than 40 mg/dL (1.0 mmol/L) for men and less than 50 mg/dL (1.3 mmol/L) for women, there is an increased risk of

heart disease that is independent of other risk factors, including the LDL-Cholesterol level.

- A typical level of HDL-Cholesterol is between 40-50 mg/dL (1.0-1.3 mmol/L) for men and between 50-59 mg/dl (1.3-1.5 mmol/L) for women and is associated with average risk of heart disease.
- Based on many epidemiologic studies, HDL-Cholesterol of 60 mg/dL (1.55 mmol/L) or higher is associated with a less than average risk of heart disease. The National Cholesterol Education Panel Adult Treatment Guidelines suggest that an HDL cholesterol value greater than 60 mg/dL is protective and should be treated as a negative risk factor. However, some recent studies suggest that high HDL-Cholesterol is not universally protective.

For children, teens and young adults:

- If HDL-Cholesterol is less than 40 mg/dL (1.04 mmol/L), there is an increased risk of heart disease that is independent of other risk factors, including the LDL- Cholesterol level.
- A level of HDL-Cholesterol between 40 and 45 mg/dL (1.04-1.17 mmol/L) is borderline.
- A level of HDL-Cholesterol greater than 45 mg/dL (1.17 mmol/L) is acceptable.

The principle method:

Directly determination of serum HDLc (high-density lipoprotein cholesterol) levels without the need for any pre-treatment or centrifugation of the sample.

The assay takes place in two steps.

1. Elimination of lipoprotein no-HDL:

$$\text{Cholesterol esters} \xrightarrow{\text{CHE}} \text{Cholesterol} + \text{Fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{CHOD}} 4-\text{Cholestenone} + H_2O_2$$

$$2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2$$

2. Measurement of HDL-Cholesterol

$$\text{Cholesterol esters} \xrightarrow{\text{CHE}} \text{Cholesterol} + \text{Fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{CHOD}} 4-\text{Cholestenone} + H_2O_2$$

$$2H_2O_2 + \text{HDAOS} + 4-\text{AA} \xrightarrow{\text{POD}} \text{Quinonimine} + 4H_2O$$

The intensity of the color formed is proportional to the HDL-Cholesterol concentration in the sample.

Procedure:

- Assay conditions:
  - Wavelength …… 500 nm.
  - Cuvette ……... 1 cm light path.

    Low level (risk factor) < 40, high level (Protective factor) ≥ 60.
  - Standard concentration = 100 mg / dl.
- Adjust the instrument (colorimeter) to zero with distilled water.
- Pipette into a cuvette:

Precipitant Reagent (HDL-Reagent)	400 µL		
Serum	200 µL		
- Mix for (10 min) at room temperature -Separate for (10 min) with centrifuge (4000 r.p.m.). Then:-			
	Blank	**Standard**	**Sample**
Working Reagent (Cholesterol Reagent)	**1000 µL**	**1000 µL**	**1000 µL**
Standard		**50 µL**	
separator			**50 µL**

- Mix and incubate 5 minutes at (37) ºC or 10 minutes at (15-25) ºC, then read the absorbance (A) at 500 nm.
- Calculation: $\frac{A^o \text{ sample}}{A^o \text{ Standard}} \times \text{Conc} =$ mg / dl