



349BCH

Triacylglycerol Estimation

Amani Alghamdi

Afnan Shaiban Alrashdi

425201430

Objectives:

The quantitative determination of the triglycerides in serum or plasma .

Introduction:

Triglycerides are a type of fat found in blood. They are a major source of energy and the most common type of fat in your body.

When eat, the body uses the calories it needs for quick energy. Any extra calories are turned into triglycerides and stored in fat cells to be used later. The excess calories are stored as fat regardless of what kind of food you eat-fat, carbohydrate, or protein. If you regularly eat more calories than you burn, you may have high triglycerides.

In normal amounts, triglycerides are important to good health. When triglyceride levels are high, it is not clear whether these high levels directly increase the risk for heart disease. But high triglycerides are often part of a group of conditions called metabolic syndrome.

Metabolic syndrome is the combination of high blood pressure, high blood sugar, too much fat around the waist, low HDL ("good") cholesterol, and high triglycerides. This syndrome does increase the risk for heart disease as well as for diabetes and stroke.

Triglycerides are measured as part of a blood test that measures the cholesterol. Normal triglyceride levels are below 150. High levels are 200 or above.

Conditions that may cause high triglycerides include:

- Obesity.
- Poorly controlled diabetes.
- An underactive thyroid (hypothyroidism).
- Kidney disease.
- Regularly eating more calories than you burn.
- Drinking a lot of alcohol.

Certain medicines may also raise triglycerides. These medicines include:

- Tamoxifen.
- Steroids.
- Beta-blockers.
- Diuretics.
- Estrogen.
- Birth control pills.

Certain types of high cholesterol and high triglycerides are caused by genetics.

High triglycerides usually do not cause symptoms.

Sometimes high triglycerides have a genetic cause. But this is not common. In this case, high triglyceride levels may occur along with visible fatty deposits under the skin called xanthomas.

In rare cases, people who have very high levels of triglycerides may develop inflammation of the pancreas (pancreatitis). This can cause sudden, severe abdominal pain, loss of appetite, nausea, vomiting, and fever.

If they have overweight, losing weight may be the best way to lower triglycerides. they may be able to lower the triglycerides by eating fewer calories and increasing the activity. Other diet changes that might help include limiting fats and sugars, and limiting or not drinking alcohol.

If increasing the activity and watching calories do not work, they may also need medicine. Medicines called statins are commonly used to lower LDL ("bad") cholesterol. But drugs called nicotinic acid (or niacin) and fibrates seem to work better for lowering triglycerides. If they have high triglycerides, high LDL cholesterol, and low HDL ("good") cholesterol, they may take more than one type of medicine that lowers cholesterol.

Triglycerides determinations are of interest in the diagnosis and treatment of atherosclerosis, poorly controlled diabetes mellitus, nephrosis, liver disease , or other diseases involving lipid metabolism.

The triglycerides (GPO) method is based on the enzymatic determination of glycerol using the enzyme glycerol phosphate oxidase (GPO) after hydrolysis by lipoprotein lipase. The principle of this method was described by Fossati¹ who coupled the reaction with the classical Trinder² reaction sequence. This single reagent procedure quantitates the total glycerides in serum including the mono and diglycerides, and the free glycerol fractions. This approach is the basis for this method.

Lipase

Triglycerides -----→ Glycerol + Fatty Acids

GK

Glycerol + ATP -----→ Glycerol-1-phosphate + ADP

GPO

Glycerol - 1 - phosphate + O₂-----→DAP + H₂O₂

POD

H₂O₂ + 4-AA +4 -Chlorophenol -----→ Quinoneimine Dye + HCL + 2H₂O

Serum triglycerides are hydrolyzed to glycerol and free fatty acids by lipase. In the presence of ATP and glycerol kinase (GK), the glycerol is converted to glycerol-1-phosphate. The glycerol-1-phosphate is then oxidized by glycerol phosphate oxidase (GPO) to yield hydrogen peroxide. The condensation of hydrogen peroxide with 4-chlorophenol and 4-aminophenazone (4-AA) in the presence of peroxidase (POD) produces a red colored quinonimine dye which absorbs at, or near 500nm. The intensity of the colored complex formed is directly proportional to the triglycerides concentration of the sample.

Materials:

Tg-Buffer Reagent (R1) , Tg-Enzyme Reagent (R1a) and Tg Standard (200mg/dl).

Spectrophotometer , cuvettes , Pipettes , constant temperature incubator set at 37C ,
Timer and water , Serum .

Method:

Pipette into clean dry test tubes..

	Blank	Standard	Test 1	Test 2
Reconstituted Reagent	2.5 ml	2.5 ml	2.5 ml	2.5 ml

Pre-warm at 37 C and add:

Standard	-----	0.025 ml	-----	-----
Sample	-----	-----	0.01ml	0.025 ml

Mix and incubate at 37 C for 10 minutes , read the absorbance of standard and Sample at 505 +_ 5 nm against blank .

Result:

Blank	0.000
stander	0.252
Test 1	0.081
Test 2	0.220

Calculation:

(Test/standard)* concentration of STD (mg/dl) = concentration in test (mg/dl) .

patient 1= (0.081/0.252)* 200 = 64.286 mg/dl

patient 2= (0.220 /0.252) * 200 = 174.6032 mg/dl

Discussion:

In normal triglyceride are important to good health . when triglyceride level are high , it is not clear whether these high level directly increase the risk for heart disease . Normal level are below 150 ,high level are 200 or above.

In this experiment the patient 1 the level of triglyceride is 64.286 mg/dl , this result in normal range . the patient 2 the level of triglyceride is 174.6032 mg/dl , this result in borderline-high rang , this patient may be the way unhealthy food and eating high calorie food and do not exercise and this lead to borderline-high of triglyceride .

Question:

Why is the triglyceride level higher in plasma than in serum ?

Because the plasma containing blood protein(Albumin - Globulin - Fibrinogen and Trumbin) , which become triglyceride.

How would the state of nutrition and general metabolic state of a person affect the serum triglyceride level?

Most food that contain carbohydrates and protein into a triglyceride because the metabolic state is controlled in the level in the body triglyceride.