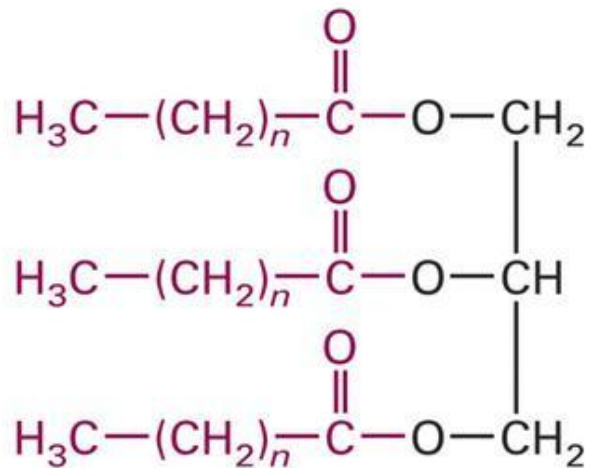


# determination of Triglyceride in Serum



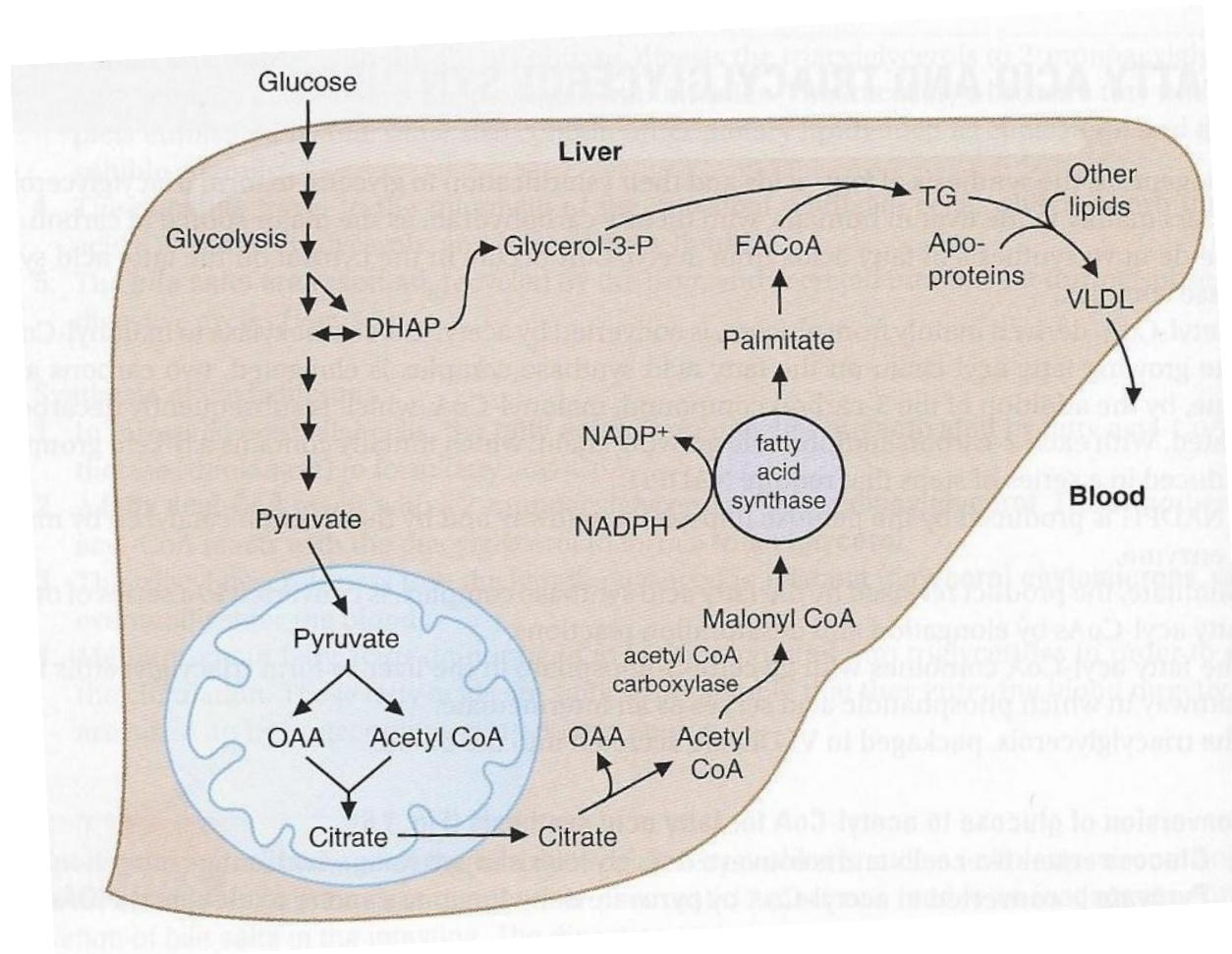
- **Triglyceride** are fatty acid esters of glycerol ,and are the main lipids in the diet . They broken down(by lipase) in the small intestine to a mixture of mono-glyceride ,fatty acid and glycerol.
- These products are absorbed and the triglycerides are re-synthesized from them in the mucosal cell. Most of these exogenous triglycerides pass into plasma as chylomicrones.

Endogenous triglycerides synthesis occur in liver from fatty acid and glycerol.

The triglycerides synthesized in this way are transported as VLDL ( Very Low Density Lipoprotein). Liver is involved in hydrolysis of triglycerides using the glycerol for glycolysis and the fatty acids for  $\beta$ -oxidation, and synthesis of most lipoproteins.

*Note: 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.*

# Synthesis of Triglyceride



*In human the synthesis of fatty acids from glucose occur mainly in Liver , fatty acids then converted to Triglyceride , packaged into VLDL, and secreted into circulation.*

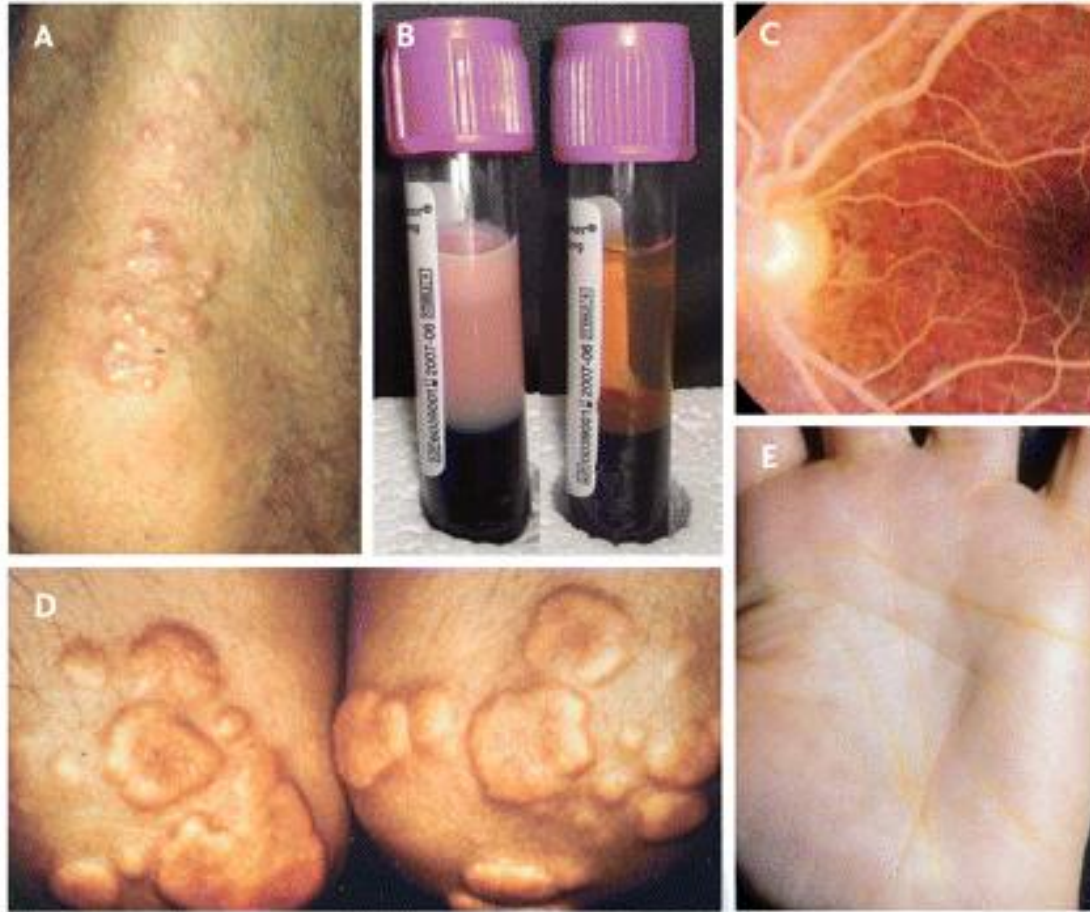
# WHY THE TEST IS PREFORMED

Elevated levels of triglycerides in plasma have been identified as risk factors related to atherosclerotic 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

- Triglyceride determinations is useful in the diagnosis of primary and secondary hyperlipoproteinemia. They are also of interest in following the course of diabetes mellitus, nephrosis, biliary obstruction, and various metabolic abnormalities due to endocrine disturbances



# Clinical manifestations of primary hypertriglyceridemia.



1. Serum of a patient with triglyceride-related acute pancreatitis.  
Left: Lipemic serum before treatment.  
Right: Clear serum after 5 days of therapy.



**A:** xanthomas (here on a patient's knee) are filled with foam cells that appear as yellow morbiliform eruptions 2–5 mm in diameter, Most often associated with markedly elevated plasma chylomicrons in cases of familial chylomicronemia (hyperlipoproteinemia type 1) or primary mixed dyslipidemia (hyperlipoproteinemia type 5), they usually occur in clusters on the skin of the trunk, buttocks or extremities.

**B:** Lipemic plasma. Whole blood has been allowed to stand at 4°C overnight. The sample on the left comes from a patient whose fasting total cholesterol result was 14.2 mmol/L and triglyceride concentration was 41.8 mmol/L. The sample on the right comes from a normolipidemic subject.

**C:** Lipemia retinalis. A milky appearance of the retinal vessels and pink retina can be seen when plasma triglyceride concentration exceeds 35 mmol/L.

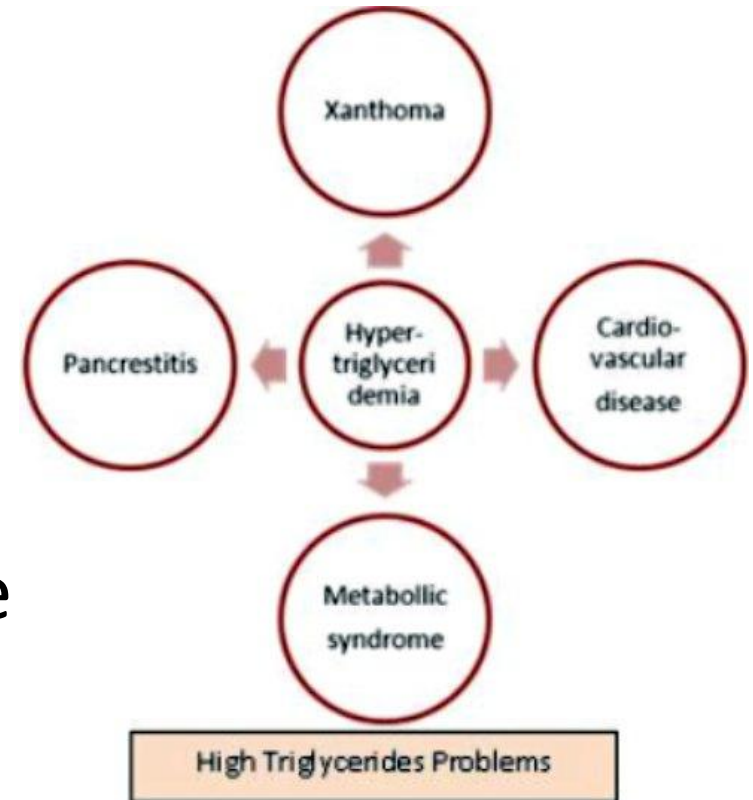
**D:** Tuberous xanthomas, filled with foam cells, appear as reddish or orange. In patients with familial dysbetalipoproteinemia (hyperlipoproteinemia type 3), they usually appear on extensor surfaces; these are on a patient's elbows.

**E:** Palmar crease xanthomas appear as yellowish deposits within palmar creases.

# primary and secondary hyperlipoproteinemia

Primary hyperlipidemia is usually due to genetic causes (such as a mutation in a receptor protein), while secondary hyperlipidemia arises due to other underlying causes such as diabetes.

Lipid and lipoprotein abnormalities are common in the general population, and are regarded as risk factor for cardiovascular disease.



# RANGE OF EXPECTED VALUES IN SERUM

Normal distributions vary with age and according to the following concentrations if exceeded, clearly indicate hyperlipidemia.

0 - 29 years .....	10 - 140 mg/dL
30 - 39 years.....	10 - 150 mg/dL
40 - 49 years.....	10 - 160 mg/dL
0 - 59 years .....	10 - 190 mg/dL



# How to Prepare for the Test

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



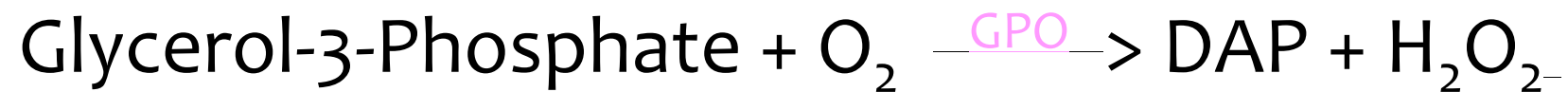
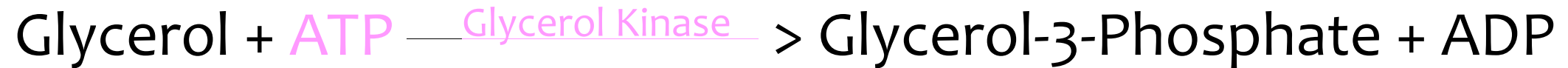
# METHOD PRINCIPLE

Standard methods for the measurement of triglycerides concentration have involved either an enzymatic or an alkaline hydrolysis to liberate glycerol.

This formulation makes use of the enzymatic hydrolysis and quantification since it is specific and not subject to interference by phospholipids

# METHOD PRINCIPLE

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



# MATERIALS

## i. CHEMICALS:

### 1. TRIGLYCERIDES BUFFER REAGENT:

- Buffer 40 mmol/L, pH-7.5
- 4- Chlorophenol 5.0 mmol/L
- Magnesium-ions 5.0 mmol/L,

### 2. TRIGLYCERIDES ENZYME REAGENT :

- ATP 3.3 mM,
- 4-Aminoantipyrine 0.7 mM,
- Glycerol-3-PhosphateOxidase 7000 U/L
- Sodium Azide 0.01%,
- Lipase 200,000 U/L,
- Glycerol Kinase 100 U/L
- peroxidase 3,000 U/L.

### 3. TRIGLYCERIDES STANDARD (200 mg/dL ):

- 2.2584 mmol/L of Glycerol with Surfactant.
- Sodium azide 0.01% Added as a preservative.

### 4. SERUM SAMPLES.

Patient should be fasting

# MATERIALS

## ii. GLASSWARE

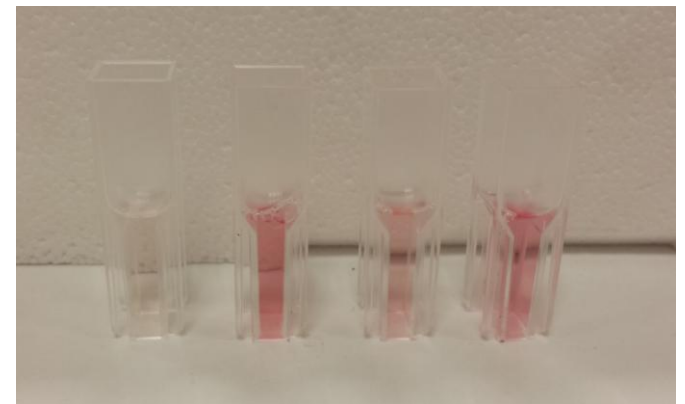
- Spectrophotometer
- Cuvettes
- Pipettes
- Constant temperature incubator set at 37 °C
- Timer
- Distilled water.

# PROCEDURE

1-Pipette into clean dry test tube

Chemical	BLANK	STANDARD	Sample 1	Sample 2
Reconstituted Reagent	1ml	1ml	1ml	1ml
Pre-warm at 37 °C and add:				
Standard	--	0.01 ml (10 $\mu$ l)	--	--
Serum Sample 1	--	--	0.01 ml (10 $\mu$ l)	--
Serum Sample 2	--	--	--	0.01 ml (10 $\mu$ l)

2- Mix and incubate at 37 °C for 10 minutes, Read the absorbance of standard and sample at 505 nm against blank.





# CALCULATIONS

A=Absorbance

## Concentration of Triglyceride in Serum Sample 1

$$= \frac{A(\text{TEST})}{A(\text{STANDARD})} \times \text{CONC. OF STD.} = \quad (\text{mg/dL})$$

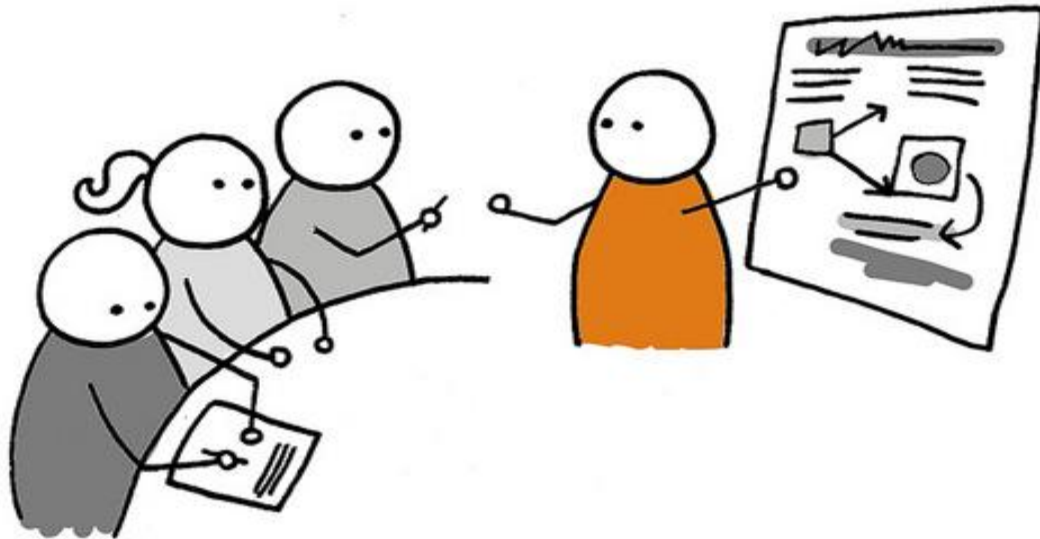
## Concentration of Triglyceride in Serum Sample 2

$$= \frac{A(\text{TEST})}{A(\text{STANDARD})} \times \text{CONC. OF STD.} = \quad (\text{mg/dL})$$

**TRIGLYCERIDES STANDARD = (200 mg/dL)**

# DISCUSSION

Comment on the concentration of Triglyceride in sample 1 and sample 2 .



# REFERENCES

- TRIGLYCERIDES (GPO) REAGENT SET from UDI .
- <http://www.ncbi.nlm.nih.gov/pubmed/8411690>
- <http://www.cmaj.ca/content/177/6/603.1/F1.expansion.html>
- **Lecture Notes: Clinical Biochemistry** Geoffrey Beckett, Simon W. Walker, Peter Rae
- **BRS Biochemistry, Molecular Biology, and Genetics**

