TRIGLYCERIDES

GPO-PAP Method

Intended Use:
Triglycerides Test reagent/kit is a medical device intended for estimation of Triglycerides in serum or plasma.

Clinical Significance
Triglycerides are a form of fatty acid esters. They are produced in the liver by binding glycerol and other fatty acids. They are transported by VLDL and LDL and act as a storage source for energy. Increased levels are found in hyperlipidemias, diabetes, nephrotic syndrome, hypothyroidism. Increased levels are risk factor for arteriosclerosis coronary disease and peripheral vascular disease. Decreased levels are found in malnutrition and hyperthyroidism.

Principle

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

Glycerol+ATP $\xrightarrow{\text{Glycerol Kinase}}$ Glycerol 3Phosphate+ADP

Glycerol 3 Phosphate $+O_2$ Phosphate $\xrightarrow{\text{GPO}}$ Dihydroxyacetone $+H_2O_2+O_2$

H_2O_2+O+4-Aminoantipyrine + Phenol $\xrightarrow{\text{Peroxidase}}$ Purple Quinoneimine dye

Intensity of the colour formed is directly proportional to the amount of triglycerides present in the sample.

Reagent Composition:

R1 Triglycerides Reagent	Lipase	≥	4KU/L
	Glycerol Kinase	≥	40KU/L
	Glycerol Phosphate Oxidase	≥	5KU/L
	Peroxidase	≥	820U/L
	4AAP	≥	1mmol/L
	Buffer	≥	20mmol/L
		P^H-7.0	
	Surfactants & Stabilizers		
Standard	200mg/dl		

Working Reagent Preparation
Reagent is ready to use.

Stability and Storage
Store at 2-8ºC
All the kit contents are stable until the expiry date stated on the label. Do not use reagents beyond the expiration date. Store the vials tightly closed protected from light and prevent contaminations during the use.

Discard if signs of deterioration appear:
- Presence of particles and turbidity.

Materials required
- Photometer or spectrophotometer with a thermostat cellcompartment set at 25/30/37ºC, capable of reading at 546nm.
- Stopwatch, strip-chart recorder or printer.
- Cuvettes with 1-cm path length
- Pipettes to measure reagent and samples.

Sample and Stability
Serum or plasma
Serum or heparinized plasma free of hemolysis.
Triglycerides is reported to be stable in the sample for 5 days when stored at 2-8^{0}C.

Assay Procedure
Pipette into clean dry test tubes labeled as Blank (B), Standard (S) and Test (T)

Addition Sequence	Blank	Std	Test
R1 Triglycerides Reagent	1000µl	1000µl	1000µl
Standard (S)	-	10µl	-
Sample	-	-	10µl

Mix well and incubate at 37^{0}C for 10 min. or at R.T. (<30^{0}C) for 15 Min. Measure the absorbance of the Standard (Abs S), and Test sample (Abs.T) against Blank, at 546 nm within 60 min.

Calculations

$$\text{Triglycerides in mg/dl} = \frac{\text{Abs of T}}{\text{Abs of S}} \times 200$$

Quality Control
To ensure adequate quality control (QC), each run should include aset of controls (normal and abnormal) with assayed values handledas unknowns.
If the values are found outside of the defined range, check the instrument, reagents and procedure.
Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable results

Linearity
This procedure is linear upto 1000 mg/dl. If values exceed this limit, dilute the serum with normal saline (NaCl 0.9%) and repeat the assay.

Reference Values

Serum/Plasma	(Suspicious)	: 150 mg/dl and above
	(Elevated)	: 200 mg/dl and above

It is recommended that each laboratory establishes its own normal range representing its patient population.

General System Parameters

Mode	End Point
Reaction	Increasing
Wavelength	546nm
Blank with	Reagent
Sample Volume	10µl
Reagent Volume	1000µl
Std Conc.	200 mg/dl
Incub. Temp.	37^{0}C
Incub. Time	10 min.
Delay Time	5 sec
Normal Range	150 – 200 mg/dl
Linearity	Up to 1000mg/dl
Unit	mg/dl

Notes
1. Fasting samples of 12 to 14 hrs. are preferred. Fatty meals and alcohol may cause elevated results. Patients should not drink alcohol for 24 hrs before the test.
2. Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.

Reference

1. Trinder,P., (1969) Ann. Clin. Biochem. 6 : 24
2. Bucolo, G., David, H., (1973) Clin. Chem. 19: 476
3. Fossati, P., Principe, L., (1982) Clin. Chem. 28 : 277

For *in vitro* Diagnostic use only.