PATHOZYME DIAGNOSTICS

LIQVIPATH TRIGLYCERIDES

(GPO – PAP Method)

CAT NO.: TGL

Reagent for quantitative estimation of Triglycerides in Serum or Plasma.

DIAGNOSTIC SIGNIFICANCE:

Normally, Triglycerides, HDL-cholesterol, Total Cholesterol are estimated, and LDL-cholesterol is calculated. These parameters represent a routine practical aspect of lipid profile which is useful in determination of risk factor or health status of a subject. Serum triglycerides estimation is an important parameter in the investigation of hyperlipoproteinaemia. Elevated levels may be found in atherosclerosis, diabetes mellitus, glycogen storage diseases like in Von Gierke's disease, secondary hyperlipoproteinaemia, alcoholism and nephrotic syndrome.

PRINCIPLE:

Lipase hydrolyses triglycerides sequentially to Di & Monoglycerides and finally to glycerol. Glycerol Kinase (GK) using ATP as P04 source converts Glycerol liberated to Glycerol-3-Phosphate (G-3-Phosphate). G-3-Phosphate Oxidase (GPO) oxidises, G-3-Phosphate formed to Dihydroxy acetone phosphate and hydrogen peroxide is formed. Peroxidase (POD) uses the hydrogen peroxide formed, to oxidise 4-Aminoantipyrine and chromogen to a purple coloured complex. The absorbance of the coloured complex is measured at 546nm (520-550 nm or with green filter) which is proportional to Triglyceride concentration.

$$\text{Triglycerides} + H_2O \xrightarrow{\text{Lipase}} \text{Glycerol} + \text{Fatty Acids}$$

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

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

$$H_2O_2 + \text{4-Aminoantipyrine} + \text{Chromogen} \xrightarrow{POD} \text{Quinoneimine} + H_2O$$

SPECIMEN COLLECTION:

Fresh, clear fasting serum with no hemolysis should be used. Heparin/ citrated plasma may be used. No other anticoagulant is suitable.

Serum levels are slightly (5mg/dl) higher than plasma levels.

PRESENTATION:

Pack Size	2 X 25 ml	3 X 50 ml	5 X 100 ml
Triglycerides Rgt.	2 X 25 ml	3 X 50 ml	5 X 100 ml
Triglycerides Std.	1 X 01 ml	1 X 02 ml	1 X 02 ml

PREPARATION OF WORKING REAGENT:

Triglycerides reagent is Ready-to-use.

REAGENT STORAGE & STABILITY:

Triglycerides reagent and standard are stable at 2-8^{0}C until the expiry date indicated on the label.

PRECAUTION:

Reagent Protected from light. Keep caped when do not use.

ASSAY PARAMETERS:

Reaction	: End point	Sample Volume	: 10 µl
Wavelength 1	: 546 nm (520-550)	Reagent Volume	: 1.0 ml
Wavelength 2	: 630 nm	Standard Conc.	: 200 mg/dl
Zero Setting	: Reagent Blank	Linearity	: 1200
Incub. Time	: 5 minutes at 37 ^{0}C	Unit	: mg/dl

PROCEDURE:

Pipette into TT	Blank	Standard	Test
Triglycerides Reagent	1.0 ml	1.0 ml	1.0 ml
Triglycerides Standard	--	10 µl	--
Sample (Test)	--	--	10 µl

Mix and incubate at 37^{0}C for 5 minutes. Read absorbance of Standard (**S**) and Test (**T**) after 5 minutes against reagent blank at 546 nm (520-550 nm or Green filter).

CALCULATION:

Triglycerides (mg/dl) = Abs T ÷ Abs S X 200

Male: 65 - 190 mg/dl	**Female**: 45 - 170 mg/dl

To convert mg/dl to mMol/L, use the following equation
mMol/L = mg/dl x 0.0114

NORMAL VALUES:

Each laboratory should establish its own normal range.

LINEARITY:

This procedure is linear up to 1200 mg/dl. For sample values higher than 1000 mg/dl, dilute the sample suitably with 0.9 % saline and repeat the assay. Apply dilution factor to obtain test results.

REFERENCES:

1. FOSSATI P., LORENZO, P.: Serum Triglycerides determined colorimeterically with an enzyme that produces hydrogen peroxide, Clin. Chem 28.2077 - 2080(1982).
2. Mc GOWAN, M. W. ARTISS, J. D. STRANBERG, D. R. ZAK, B. A. Peroxidase coupled method for the colorimetric determination of serum Triglycerides, Clin. Chem.29, 538-542(1983).

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