

(GPO/PAP METHOD)

Code: 11032/33/34/35 (5x10/2x50/2x100/5x100 ml)

(For the Analyser / Colorimetric Estimation of Triglycerides in Serum)

In VITRO USE Only.

SUMMARY & EXPLANATION OF TEST:

Triglycerides circulate in blood as complexes with protein molecules called lipoproteins. Triglycerides reach maximum level in blood approximately 4 to 6 hours post prandial. Elevated levels of both cholesterol and triglycerides in blood have been identified as risk factors related to atherosclerotic disease. The levels of cholesterol and triglycerides can vary independently, therefore evaluation of hyperlipidemia includes determinations of both cholesterol and triglycerides.

Triglycerides are usually assayed either by chemical methods or enzymatic methods. The chemical methods involve solvent extraction; chemical hydrolysis to release glycerol; oxidation of glycerol to form aldehyde which is then quantitated by coupling it with chromogens. These chemical methods are cumbersome, time consuming and are reliable only in expert hands. In contrast the enzymatic methods are simple, and easy to perform.

PRINCIPLE:

Triglycerides are hydrolyzed by lipase to glycerol and free fatty acids. Glycerol is phosphorylated by ATP in the presence of glycerolkinase (GK) to Glycerol-3-Phosphate (G-3-P) which is oxidized by the enzyme Glycerol-3-Phosphate Oxidase (G-P-O) producing hydrogen peroxide. Hydrogen peroxide so formed reacts with 4-aminoantipyrine and ESPAS in the presence of the enzyme peroxidase (POD) to produce a Brown Colour Complex. The intensity of the color developed is proportional to the triglycerides concentration.

Lipase

Triglycerides + H₂O Glycerol + Fatty acids.

Glycerol + ATP G-3-P + ADP

G-3-P+O₂ $\xrightarrow{\text{H}_2\text{O}_2}$ \Rightarrow Dihydroxyacetone Phosphate.

 $H_2O_2 + ESPAS + \cancel{A}$ -AAP Brown Color + H_2O

REAGENTS:

1. Enzyme Reagent 5x10 2x50 2x100 5x100ml

2. Standard (200 mg%) 1.0 1.0 1.0 2x1.0ml

The reagents are ready to use and usable upto the expiration date when stored at 2-8°C.

SAMPLE:

Serum / Plasma

EXPECTED RANGE:

Serum Triglycerides : Upto 150 mg%

LINEARITY:

Upto 1000 mg%

INSTRUCTIONS:

- 1. Avoid use of detergents for cleaning glassware. The presence of traces of detergent impurities interferes in the final color development.
- 2. All reagents are ready to use. Discard upon turbidity. Slight pink color (up to 0.15 Abs) does not effect the performance of the reagents.

DIRECTIONS FOR USE ON ANALYSERS:

Reaction Type : End point with std.

Wave Length : 546 nm / green filter

Incubation Temp : 37°C
Incubation Time : 10 min.
Standard : 200 mg%
Linearity : 1000 mg%
Unit : mg%

PROCEDURE:

Pipette into clean dry tubes labeled Blank (B), Standard (S), and Test (T).

В	S	Т
1.0ml	1.0ml	1.0ml
-	0.01ml	-
-	-	0.01ml
	1.0ml -	1.0ml 1.0ml - 0.01ml

Mix well and incubate for 10 minutes at 37°C. Read absorbance of Standard and Test against Blank on photocolorimeter at 546 nm/green filter.

CALCULATIONS:

 $mMol/L = mg\% \times 0.0113$

 $\label{eq:A of (T)} \mbox{Triglycerides conc. in mg% = } \begin{tabular}{ll} A of (T) \\ & x 200 \mbox{ (Std. Conc)} \\ A of (S) \end{tabular}$ SI conversion factor

NOTES:

- ★ Due to variations in inter laboratory assay conditions, instruments and demography, it is recommended that each laboratory should establish its own normal range. To ensure adequate quality control, each run should include a normal and abnormal assayed controls. The assigned value of the control must be confirmed by this methodology.
- ★ Final diagnosis should be based on a co-relation of test results with other clinical observations / Diagnostic tools.

BIBLIOGRAPHY:

- 1. Bucolo G., David M. "Clin. Chem." 19.476 (1973)
- 2. Werner M., Gabrielson D.G., Eastman G. "Clin. Chem." 27.268 (1981)

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