TRIGLYCERIDES KIT

INTRODUCTION & PRINCIPLE:

Triglycerides are esters of fatty acids and are hydrolyzed to glycerol and free fatty acids. Triglyceride determinations when performed in conjunction with other lipid assays are 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.

The enzymatic reaction sequence employed in the assay of Triglycerides is as follows:
Glycerol released from Triglycerides after hydrolysis by lipoprotein lipase, is transformed by Glycerol kinase into Glycerol-3-phosphate which is oxidized into dihydroxyacetone phosphate and hydrogen peroxide. In the presence of peroxidase, the Hydrogen peroxide oxidizes the chromogen to form purple Quinoneimine, whose intensity of colour is proportional to the concentration of Triglycerides in the sample.

REAGENTS & STABILITY:

1. Triglyceride Reagent : Ready – To – Use
2. Triglyceride Standard : Ready – To – Use

Equilibrate reagent at 37^0 C before adding sample/standard

Avoid Contamination of the Ready - To - Use Reagents. Always use fresh pipette tips. Keep always the caps tightly closed.

SPECIMEN COLLECTION:

1. Fresh, clear, non-hemolyzed serum from fasting patients is recommended.
2. Triglycerides in serum is stable for three days when stored at 2^0 to 8^0 C.
3. Prolonged storage of the samples at Room Temperature is not recommended since other glycerol containing compounds may hydrolyze, releasing free glycerol, with an apparent increase in total Triglyceride content.
4. Blood collection devices lubricate with glycerin (glycerol) should not be used.

INTERFERING SUBSTANCES:

Heparin, EDTA, fluoride, oxalate and citrate may inhibit Triglycerides and should be avoided. Lipemic or grossly icteric samples will cause falsely elevated results, consequently a serum blank should be run. Add 0.01 ml (10 ul) of sample to 1.0 ml of water, mix and read against water blank at 540 nm. Subtract this absorbance to yield a corrected reading. Young et. al. give a comprehensive review of drug interferences.

PROCEDURE:

METHOD	: End Point
WAVE LENGTH	: 505 nm
TEMPERATURE	: 37^0 C
CUVETTE	: 10 mm path length
MEASUREMENT	: Against blank
INCUBATION	: 15 mins. at R.T.
STANDARD	: 200 mg/dl (refer the vial Label)

Pipette into cuvettes	B	S	T
Reagent	1000 ul	1000 ul	1000 ul
Sample/ Standard	-	10 ul	10 ul

Mix & incubate for 15 mins. at R.T. and 10 mins.at 37^0 C and read the absorbance of the Sample (A – sample) and Standard (A – Standard) against Blank at 505 nm, within 30 minutes against reagent blank.

CALCULATIONS:

$$\frac{\text{Abs (Sample)}}{\text{Abs (Standard)}} \times \text{Concentration of Standard (mg/dl)} = \text{Concentration of Triglycerides (mg/dl)}$$

EXPECTED VALUES : 36 – 165 mg/dl

LINEARITY : 1000 mg/dl

REFERENCES:

1. Bucolo G., David M "Clin. Chem." 19, 476 (1973)
2. Wemer M., Gabriel son D.G. Eastman G. 'Clin. Chem." 27, 268. (1981).

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